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Title 21—FOOD AND DRUGS

Chapter I—Food and Drug Administration, Department of Health, Education, and Welfare

SUBCHAPTER C—DRUGS

TESTS AND METHODS OF ASSAY AND CERTIFICATION OF ANTIBIOTIC DRUGS SUBJECT TO DRUG AMENDMENTS OF 1962

The Commissioner of Food and Drugs has evaluated the views and comments received in response to the notice of proposed rule-making published in the FEDERAL REGISTER of May 2, 1963 (28 F.R. 4411–4429, inclusive), and has concluded that the following regulations should issue for the certification of those antibiotic drugs subject to the Drug Amendments of 1962 (76 Stat. 785–787; Public Law 87–781). Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 507, 59 Stat. 463, as amended; 21 U.S.C. 357), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (21 CFR 2.90; 29 F.R. 471), the following amendments are ordered: [1]

PART 146—GENERAL REGULATIONS FOR THE CERTIFICATION OF ANTIBIOTIC AND ANTIBIOTIC-CONTAINING DRUGS

1. Section 146.1 is amended as follows:

a. By revising the section heading to read:

§ 146.1 Definitions and interpretations applicable to all certifiable antibiotic drugs.

b. By adding to paragraph (a) the following new subparagraphs:

(8) Each of the antibiotic substances produced by the growth of Streptomyces canus, and each of the same substances produced by any other means, is a kind of amphomycin.

(9) Each of the antibiotic substances produced by the growth of Streptomyces nodosus, and each of the same substances produced by any other means, is a kind of amphotericin.

(10) Each of the antibiotic substances produced by the growth of Bacillus polymyxa var. colistinus, and each of the same substances produced by any other means, is a kind of colistin.

(11) Each of the antibiotic substances produced by the growth of Streptomyces orchidaceus, and each of the same substances produced by any other means, is a kind of cycloserine.

(12) Each of the antibiotic substances produced by the growth of Streptomyces erythreus, and each of the same substances produced by any other means, is a kind of erythromycin.

(13) Each of the antibiotic substances produced by the growth of Bacillus brevis, and each of the same substances produced by any other means, is a kind of gramicidin.

(14) Each of the antibiotic substances produced by the growth of Penicillium patulum or Penicillium griseofulvum, and each of the same substances produced by any other means, is a kind of griseofulvin.

(15) Each of the antibiotic substances produced by the growth of Streptomyces kanamyceticus, and each of the same substances produced by any other means, is a kind of kanamycin.

(16) Each of the antibiotic substances produced by the growth of Streptomyces fradiae, and each of the same substances produced by any other means, is a kind of neomycin.

(17) Each of the antibiotic substances produced by the growth of Streptomyces niveus (known also as Streptomyces spheroides), and each of the same substances produced by any other means, is a kind of novobiocin.

(18) Each of the antibiotic substances produced by the growth of Streptomyces noursei, and each of the same substances produced by any other means, is a kind of nystatin.

(19) Each of the antibiotic substances produced by the growth of Streptomyces antibioticus, and each of the same substances produced by any other means, is a kind of oleandomycin.

(20) [Reserved]

(21) Each of the antibiotic substances produced by the growth of Streptomyces rimosus, and each of the same substances produced by any other means, is a kind of oxytetracycline.

(22) Each of the antibiotic substances produced by the growth of Streptomyces rimosus var. paromomycinus, and each of the same substances produced by any other means, is a kind of paromomycin.

(23) Each of the antibiotic substances produced by the growth of Bacillus polymyxa, and each of the same substances produced by any other means, is a kind of polymyxin.

(24) Each of the antibiotic substances produced by the growth of Nocardia lurida, and each of the same substances produced by any other means, is a kind of ristocetin.

(25) Each of the mixtures of antibiotic substances produced by the growth of Bacillus brevis, and each of the same mixtures of substances produced by any other means, is a kind of tyrothricin.

(26) Each of the antibiotic substances produced by the growth of Streptomyces orientalis, and each of the same substances produced by any other means, is a kind of vancomycin.

(27) Each of the antibiotic substances produced by the growth of Streptomyces puniceus or Streptomyces floridae or Actinomyces vinaceus, and each of the same substances produced by any other means, is a kind of viomycin.

c. By adding to paragraph (b) the following new subparagraphs:

(10) The term "amphomycin master standard" means a specific lot of amphomycin designated by the Commissioner as the standard of comparison in determining the potency of the amphomycin working standard.

(11) The term "amphotericin A master standard" means a specific lot of amphotericin A designated by the Commissioner as the standard of comparison in determining the potency of the amphotericin A working standard. The term "amphotericin B master standard" means a specific lot of amphotericin B designated by the Commissioner as the standard of comparison in determining the potency of the amphotericin B working standard.

(12) The term "colistin master standard" means a specific lot of colistin designated by the Commissioner as the standard of comparison in determining the potency of the colistin working standard.

[1] Certain portions of the proposal will be covered by a subsequent order.

(13) The term "colistimethate master standard" means a specific lot of colistimethate designated by the Commissioner as the standard of comparison in determining the potency of the colistemethate working standard.

(14) The term "cycloserine master standard" means a specific lot of cycloserine designated by the Commissioner as the standard of comparison in determining the potency of the cycloserine working standard.

(15) The term "erythromycin master standard" means a specific lot of erythromycin designated by the Commissioner as the standard of comparison in determining the potency of the erythromycin working standard.

(16) The term "gramicidin master standard" means a specific lot of gramicidin designated by the Commissioner as the standard of comparison in determining the potency of the gramicidin working standard.

(17) The term "griseofulvin master standard" means a specific lot of griseofulvin designated by the Commissioner as the standard of comparison in determining the potency of the griseofulvin working standard.

(18) The term "kanamycin master standard" means a specific lot of kanamycin designated by the Commissioner as the standard of comparison in determining the potency of the kanamycin working standard.

(19) The term "neomycin master standard" means a specific lot of neomycin designated by the Commissioner as the standard of comparison in determining the potency of the neomycin working standard.

(20) The term "novobiocin master standard" means a specific lot of novobiocin designated by the Commissioner as the standard of comparison in determining the potency of the novobiocin working standard.

(21) The term "nystatin master standard" means a specific lot of nystatin designated by the Commissioner as the standard of comparison in determining the potency of the nystatin working standard.

(22) The term "oleandomycin master standard" means a specific lot of oleandomycin designated by the Commissioner as the standard of comparison in determining the potency of the oleandomycin working standard.

(23) The term "oxytetracycline master standard" means a specific lot of oxytetracycline designated by the Commissioner as the standard of comparison in determining the potency of the oxytetracycline working standard.

(24) The term "paromomycin master standard" means a specific lot of paromomycin designated by the Commissioner as the standard of comparison in determining the potency of the paromomycin working standard.

(25) The term "polymyxin B master standard" means a specific lot of polymyxin B designated by the Commissioner as the standard of comparison in determining the potency of the polymyxin B working standard.

(26) The term "ristocetin A master standard" means a specific lot of ristocetin A designated by the Commissioner as the standard of comparison in determining the potency of the ristocetin A working standard. The term "ristocetin B master standard" means a specific lot of ristocetin B designated by the Commissioner as the standard of comparison in determining the potency of the ristocetin B working standard.

(27) The term "vancomycin master standard" means a specific lot of vancomycin designated by the Commissioner as the standard of comparison in determining the potency of the vancomycin working standard.

(28) The term "viomycin master standard" means a specific lot of viomycin designated by the Commissioner as the standard of comparison in determining the potency of the viomycin working standard.

2. In § 146.3 *Certification,* paragraph (c) is amended by changing the phrase "in Parts 141a to 141e and § 147.2 of this chapter" to read "issued under this chapter."

3. The introduction to § 146.10 is changed to read:

§ 146.10 Antibiotics for export.

Drugs containing antibiotic substances subject to certification under section 507 of the act and intended for export will be certified notwithstanding failure to meet the labeling requirements of the applicable monographs if the labeling used for such drugs complies with the following conditions:

* * * * *

4. Section 146.12 *Antibiotic drugs exempted from certification requirements under section 507(c) of the Federal Food, Drug, and Cosmetic Act* is amended in the following respects:

a. By changing the first sentence of paragraph (a) to read:

(a) Section 507(a) of the Federal Food, Drug, and Cosmetic Act directs the Secretary of Health, Education, and Welfare to promulgate regulations to provide for the certification of batches of drugs composed wholly or partly of any kind of antibiotic substance. * * *

b. By changing the item "Antibiotics for fish diseases" in paragraph (b)(3) to read:

Antibiotics for diseases of fish not intended for use as food.

5. Section 146.22 *Exemptions for manufacturing use* is amended by changing paragraph (a) to read:

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of any certifiable antibiotic drug subject to the regulations in this chapter that is packed in containers of not less than 10,000,000 units of penicillin or 10 grams each of one of the other antibiotic drugs shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in the establishment where it is so used, from the requirements of section 502(l) of the act, if it conforms to the standards prescribed therefor by the section of the regulations in this chapter which is specifically applicable to such other drug, if the label of each container bears the batch mark of the drug, the number of units or grams per package, and the date on which the latest assay of the drug was completed, and if the person who introduced each shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for manufacturing use in such establishment.

6. Section 146.24 is amended as follows:

a. By changing the section heading and the introduction to the section to read:

§ 146.24 Antibiotic powders for diagnostic use.

Dry powders of antibiotic substances, with or without suitable buffer substances and diluents, packaged for dispensing and intended for use solely in laboratory procedures in connection with the diagnosis or treatment of disease and conspicuously so labeled shall be exempt from the certification requirements of sections 502(l) and 507 of the act if they comply with all the following conditions:

b. By changing paragraph (a) to read:

(a) The potency and moisture content of the antibiotic used in the manufacture of the powder and the moisture content of the powder comply with the standards prescribed for the antibiotic by the specific regulations issued in this chapter.

7. Section 146.25 *Antibiotic drugs for use in medicated animal feed* * * * is amended by changing the phrase "regulations in this chapter" in the introduction to the section to read: "regulations in Parts 146a, 146b, 146c, 146d, and 146e of this chapter".

8. Title 21 is amended by adding thereto the following new parts:

PART 148—ANTIBIOTIC DRUGS SUBJECT TO CERTIFICATION UNDER THE DRUG AMENDMENTS OF 1962

Sec.
148.1 Definitions and interpretations.
148.2 Packaging requirements.
148.3 Labeling requirements.
148.4 Requests for certification; check tests and assays; samples.
148.5 Fees.

AUTHORITY: The provisions of this Part 148 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148.1 Definitions and interpretations.

The definitions and interpretations of terms found in § 146.1 of this chapter apply also to this Part 148 and to Parts 148a–148z of this chapter.

§ 148.2 Packaging requirements.

Each drug described in Parts 148a to 148z, inclusive, of this chapter shall be packaged in immediate containers that comply with the requirements described in Parts 146a to 146e, inclusive, of this chapter for the immediate containers used to package the same dosage form unless otherwise required for the drug by specific regulations.

§ 148.3 Labeling requirements.

(a) If it is packaged for dispensing:

(1) It shall be labeled in accordance with the requirements prescribed by § 1.106(b) of this chapter, issued under section 502(f) of the act, unless the regulations pertaining to such drug specifically exempt it from such requirements.

(2) Its labeling shall bear any additional information required for the drug by specific regulations.

(3) Each package shall bear on its outside wrapper or container and the immediate container an expiration date prescribed for the drug by specific regulations, except that the date may be used that is 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such period of time. If the manufacturer or repacker of the drug has been exempted from the certification requirements, such date shall be the number of months after the month during which the batch was last assayed and released by the manufacturer or repacker. If an expiration date is used that is longer than the minimum date provided for the drug by specific regulations, it may be used only if the manufacturer has submitted information to the Commissioner adequate to prove that the drug is stable for such time.

(b) If it is packaged solely for manufacturing use or for repacking, each package shall bear on its outside wrapper or container and the immediate container, the following:

(1) The number of units or micrograms of activity per milligram or per gram, and the number of grams of kilograms in the immediate container.

(2) The batch mark.

(3) The statement "Caution: Federal law prohibits dispensing without prescription."

(4) The statement "For manufacturing use," "For repacking," or "For manufacturing use or repacking," and, if it is not sterile, the statement "nonsterile."

(5) The required expiration date.

§ 148.4 Requests for certification; check tests and assays; samples.

In addition to complying with the requirements of § 146.2 of this chapter, a person who requests certification or check tests and assays of a batch shall submit with his request the following information and samples:

(1) The batch mark of the drug.

(2) The quantity of each ingredient used in making the batch.

(3) The size of the batch, including the number of containers of each size in the batch.

(4) The date of the latest assay of the batch.

(5) The results of tests and assays made by or for him on the batch as required for the drug by specific regulations.

(6) The batch mark(s) of the antibiotic(s) used in making the batch.

(7) Unless previously submitted, the results of tests and assays made by or for him on the antibiotic(s) used in making the batch as required by specific regulations.

(8) The number of accurately representative samples that are required for the batch by specific regulations: In the case of drugs such as dry powders, solutions, ointments, and suspensions, the sample shall be collected by taking single immediate containers, before or after labeling, at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal. In no case, however, shall more than 5,000 immediate containers have been packaged during each such interval of sampling, except for a sample collected for sterility testing. In the case of drugs such as tablets or other such unit dosage forms, the sample shall be collected by taking single tablets at such intervals throughout the entire time of tableting the batch that the quantities tableted during the intervals are approximately equal. In no case, however, shall more than 5,000 tablets have been tableted during each interval of sampling, except for a sample collected for time of disintegration. If the person who packages the tablets into dispensing-size containers is not the manufacturer, such sample shall be collected throughout the entire time of packaging the batch into such containers.

(9) Unless previously submitted, the number of accurately representative samples that are required for the antibiotic(s) used in making the batch by the regulations specifically relating to such drug.

(10) In connection with contemplated requests for certification of repacked batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so repackaged or used may request the Commissioner to make check tests and assays on a representative sample of such batch. From the information submitted may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

§ 148.5 Fees.

The fee for the services rendered with respect to each batch certified under the regulations in Parts 148a–148z, inclusive, of this chapter shall accompany the request for certification, or the request for check tests and assays, unless such fee is covered by an advance deposit maintained in accordance with § 146.8(d) of this chapter. Such fee shall be that required for the drug by the specific regulations, plus an additional 30 percent of whatever that fee may be. Also, if the Commissioner considers that investigations other than examination of such samples are necessary to determine whether or not such batch complies with the requirements of § 146.3 of this chapter for the issuance of a certificate, the fee shall include the cost of such investigations.

PART 148a—AMPHOMYCIN

Sec.

148a.1 Calcium amphomycin.
148a.2 Calcium amphomycin-neomycin sulfate-hydrocortisone acetate cream.

AUTHORITY: The provisions of this Part 148a issued under sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357.

§ 148a.1 Calcium amphomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium amphomycin is the calcium salt of amphomycin. It is so purified and dried that:

(i) Its potency is not less than 868 micrograms of amphomycin per milligram.

(ii) Its moisture content is not more than 10 percent.

(iii) Its pH in a 2 percent aqueous suspension is not less than 6.0 and not more than 7.5.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, and pH.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1 of this chapter.

(ii) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*a*) Make nutrient agar for the base layer as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.0 after sterilization.

(*b*) Make nutrient agar for the seed layer as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.0 after sterilization.

In lieu of preparing the media from the individual ingredients specified in subsections (*a*) and (*b*) of this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Preparation of suspension.* The test organism is *micrococcus flavus* (ATCC 14452). Maintain this organism on slants of nutrient agar prepared as described in subdivision (ii) (*b*) of this subparagraph and to which has been added 1 milligram of neomycin sulfate per milliliter of agar. Wash the organism from the agar slant with 3 milli-

liters of sterile U.S.P. saline T.S. onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the same type of agar used for the slants. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C. to 35° C., and then wash the resulting growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S. If an aliquot of this bulk suspension, when diluted 1:35 in the saline solution, gives 25 percent light transmission in a suitable photoelectric colorimeter with a 580-millimicron filter and a test tube with an inside diameter of 13 millimeters as an absorption cell, the bulk suspension is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution, so that an aliquot of the adjusted suspension diluted 1:35 gives 25 percent light transmission. The adjusted bulk suspension, and not the 1:35 dilution of it, is used in preparing the seed layer. Incubate seeded plates to determine the quantity of the adjusted bulk suspension (usually 0.5 millimeter) that should be added to each 100 milliliters of agar to give zones of inhibition of appropriate size.

(iv) *Preparation of plates.* Add 21 milliliters of the base layer agar prepared as directed in subdivision (ii) (*a*) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow it to harden. Use the plates the same day they are prepared. Add the predetermined aliquot of the adjusted bulk suspension to the agar described in subdivision (ii) (*b*) of this subparagraph which has been melted and cooled to 48° C. Mix the seeded agar thoroughly and add 4 milliliters to each of the plates containing 21 milliliters of the uninoculated agar described in subdivision (ii) (*a*) of this subparagraph. Tilt the plates to spread the agar evenly over the surface. Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on 2.8-centimeter radius.

(v) *Working standard.* Dry an appropriate amount of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters of mercury or less. Determine the dry weight and dissolve the dried standard in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock standard solution of 100 micrograms per milliliter. This should be allowed to stand overnight at room temperature in order to allow complete solution to take place. Store this stock standard solution in a refrigerator, and use for no longer than 14 days.

(vi) *Standard curve.* Prepare the daily standard curve by further diluting the 100 microgram per milliliter stock standard solution in sufficient 0.1*M* potassium phosphate buffer, pH 8, to obtain concentrations of 6.4, 8.0, 10.0, 12.5 and 15.6 micrograms per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution except the 10.0 micrograms per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates fill three cylinders with the 10.0 micrograms per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 10.0-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 10.0 micrograms per milliliter concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the 10.0 micrograms per milliliter concentration. The average of the 36 readings of the 10.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 10.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 8.0-microgram concentration the average reading of the 36 readings of the 10.0 micrograms per milliliter concentration is 16.5 millimeters and the average of the 10.0 micrograms per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 8.0 micrograms per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 10.0 micrograms per milliliter concentration, on 2-cycle semilog paper using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 10.0 micrograms per milliliter standard;

a, b, d, e = Corrected average values for the 6.4, 8.0, 12.5, and 15.6 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(vii) *Preparation of sample.* Dissolve the sample to be tested in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to make a convenient stock solution. Further dilute in buffer to a final concentration of 10.0 micrograms per milliliter (estimated).

(viii) *Plate assay.* Use three plates for each sample. Fill three cylinders on each plate with the 10.0 micrograms per milliliter standard and three cylinders with the 10.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition. Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives a larger average zone size than the average of the standard, add the difference between them to the 10.0-microgram zone size of the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 10.0-microgram value on the curve. From the curve, read the potencies corresponding to these corrected values.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a suspension prepared by adding 20 milligrams per milliliter.

§ 148a.2 Calcium amphomycin-neomycin sulfate-hydrocortisone acetate cream.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Calcium amphomycin-neomycin sulfate-hydrocortisone acetate cream is calcium amphomycin, neomycin sulfate, and hydrocortisone acetate in a suitable and harmless water-soluble cream base containing suitable buffers, emulsifiers, and preservatives. Each gram contains 5 milligrams of amphomycin, 3.3 milligrams of neomycin, and 10 milligrams of hydrocortisone acetate. The calcium amphomycin used in making the cream conforms to the standards prescribed by § 148a.1(a)(1). The neomycin sulfate used conforms to the standards prescribed by § 1481.1(a)(1) (i), (v), (vi), and (vii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium amphomycin used in making the batch for potency, moisture, and pH.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for amphomycin potency and neomycin sulfate potency.

(ii) Samples required:

(*a*) Calcium amphomycin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) Neomycin sulfate used in making the batch; 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) Fees. $4.00 for each immediate container in the samples submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container in

the sample submitted in accordance with subparagraph (3) (ii) (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Amphomycin content.* Proceed as directed in § 148a.1(b)(1), except in lieu of the directions in § 148a.1(b)(1)(vii), prepare the sample as follows: Accurately weigh approximately 2 grams, and place in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a final volume of 200 milliliters. Using a high-speed blender, blend the mixture for 2 minutes and then make the proper estimated dilutions using 0.1*M* potassium phosphate buffer, pH 8.0. Its content of amphomycin is satisfactory if it contains not less than 90 percent and not more than 140 percent of the number of milligrams of amphomycin per gram that it is represented to contain.

(2) *Neomycin content.* Blend the sample as described in subparagraph (1) of this paragraph and, making the proper estimated dilutions in pH 8.0 buffer, proceed as directed in § 148i.1(b)(1) of this chapter. Its content of neomycin is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin per gram that it is represented to contain.

PART 148b—AMPHOTERICIN

Sec.
148b.1 Amphotericin B.
148b.2 Amphotericin B intravenous.

AUTHORITY: The provisions of this Part 148b issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148b.1 Amphotericin B.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH. 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethyl formamide and dimethyl sulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 15 percent of amphotericin A.

(iii) It is nontoxic.

(iv) Its moisture content is not more than 5.0 percent.

(v) Its pH in a 3 percent aqueous suspension is not less than 6.0 and not more than 8.0.

(vi) It contains not more than 3.0 percent residue on ignition.

(vii) It exhibits absorption maxima at 362, 381, and 405 millimicrons when dissolved in dimethyl sulfoxide and diluted with absolute methyl alcohol.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3(b) of this chapter, each package shall bear on its label the statements "Store below 10° C." and "Protect from light and moisture." Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, amphotericin A content, toxicity, moisture, pH, residue on ignition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(4) *Fees.* $4.00 for each package in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Plate assay*—(a) *Cylinders (cups).* Use cylinders described in § 141a.1 of this chapter.

(b) *Culture media.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F.

(1) Make nutrient agar for preparing the inoculated agar plates as follows:

Peptone	9.4 gm.
Yeast extract	4.7 gm.
Beef extract	2.4 gm.
Sodium chloride	10.0 gm.
Dextrose	10.0 gm.
Agar	23.5 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.0 to 6.2 after sterilization.

(2) Make nutrient agar for carrying the organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(3) Make nutrient broth for preparing an inoculum of the test organism:

Peptone	10.0 gm.
Dextrose	20.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 5.6 to 5.7 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(c) *Working standard.* Dry approximately 30 milligrams of the amphotericin B working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, and dissolve the dried standard in sufficient dimethyl sulfoxide to give a stock solution containing 1,000 micrograms of amphotericin B per milliliter. This solution should be used for 1 day only.

(d) *Preparation of sample.* Dissolve the sample in sufficient dimethyl sulfoxide to give a convenient stock solution. Further dilute with dimethyl sulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute to a concentration of 1.0 microgram of amphotericin B per milliliter (estimated) using 0.2*M* potassium phosphate buffer, pH 10.5 (dipotassium phosphate, 35 grams; 10*N* sodium hydroxide, 2 milliliters). Make to 1 liter, using distilled water.

(e) *Test organism.* The test organism is Saccharomyces cerevisiae (ATCC 9763 or ATCC 2601 no vitamin requirement), which is maintained on slants of agar described in subsection (b)(2) of this subdivision and transferred once a week. After transfer, the culture is incubated at 37° C. for 24 hours and then kept refrigerated. Prepare the organism suspension by either of the following methods:

(1) Inoculate 100 milliliters of the broth medium described under subsection (b)(3) of this subdivision with a loopful of growth from the agar slant. Incubate the broth for 16 hours to 18 hours at 37° C. This broth culture may be used for several weeks if kept refrigerated.

(2) Wash the organism from the agar slant with 3 milliliters of sterile distilled water onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in subsection (b)(2) of this subdivision. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C., and then wash the resulting growth from the agar surface with about 30 milliliters of sterile distilled water. This suspension may be used for several weeks if kept refrigerated. Dilute an aliquot of the bulk suspension 1:45 with sterile distilled water and measure its light transmission in a suitable photoelectric colorimeter with a 580-millimicron filter and a test tube with an inside diameter of 13 millimeters, as an absorption cell. The bulk suspension is ready for use when an aliquot diluted 1:45 gives a light transmission of 25 percent. If necessary, adjust the bulk suspension by dilution until an aliquot diluted 1:45 gives 25 percent light transmission. At the time of inoculation dilute the adjusted bulk suspension 1:10 with sterile distilled water. Employ test plates to determine the quantity of this diluted suspension (usually about 0.7 milliliter) that should be added to each 100 milliliters of agar to give clear inhibitory zones of appropriate size. If the broth culture is used, employ test plates similarly (usually about 2.5 milliliters per 100 milliliters of agar).

(f) *Preparation of plates.* Melt a sufficient amount of the agar described in subsection (b)(1) of this subdivision, cool to 48° C., add the proper amount of the test organism as described in subsection (e) of this subdivision, and mix thoroughly. Add 8 milliliters of this inoculated agar to each Petri dish (100 millimeters x 20 millimeters, with flat bottom). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(g) *Standard curve.* Dilute aliquots of the working stock solution with dimethyl sulfoxide to give concentrations of 40.0, 28.4, 20.0, 14.2, and 10.0 micrograms per milliliter, respectively. Dilute one volume of each of these solutions with 19 volumes of 0.2*M* potassium phosphate buffer, pH 10.5, to give final concentrations of 2.0, 1.42, 1.0, 0.71, and 0.5 microgram per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution,

except the 1.0 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be thirty-six 1.0-microgram determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 37° C., and measure the diameter of each zone of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.71 microgram per milliliter concentration the average of the 36 readings of the 1.0 microgram per milliliter concentration is 16.0 millimeters and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 15.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 0.71 microgram per milliliter concentration of these same three plates is 15.0 millimeters, the corrected value is then 15.2 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L=Calculated zone diameter for the lowest concentration of the standard curve;

H=Calculated zone diameter for the highest concentration of the standard curve;

c=Average zone diameter for the 36 readings of the 1 microgram per milliliter standard;

a, b, d, e=Corrected zone diameters for the 0.5, 0.71, 1.42, and 2.0 microgram per milliliter solutions, respectively.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram per milliliter standard and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 37° C., and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the curves read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay*—(a) *Culture media.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F.

(*1*) Make yeast-beef agar for carrying the organism as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water to make	1,000.0 ml.

pH 6.6 after sterilization.

(*2*) Make yeast-beef broth for preparing an inoculum of the test organism:

Beef extract	1.5 gm.
Yeast extract	6.5 gm.
Peptone	5.0 gm.
Trypton	10.0 gm.
Dextrose	11.0 gm.
Sodium chloride	3.5 gm.
Dipotassium phosphate	3.68 gm.
Monopotassium phosphate	1.32 gm.
Distilled water to make	1,000.0 ml.

pH 6.6 after sterilization.

(*3*) Make yeast-beef assay broth:

Beef extract	1.5 gm.
Yeast extract	1.5 gm.
Peptone	5.0 gm.
Dextrose	11.0 gm.
Sodium chloride	3.5 gm.
Dipotassium phosphate	3.68 gm.
Monopotassium phosphate	1.32 gm.
Distilled water to make	1,000.0 ml.

pH 6.6 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*b*) *Working standard and standard solutions.* Use the working standard described in subdivision (i) (*c*) of this subparagraph. Dissolve in dimethyl sulfoxide to obtain a concentration of 2 milligrams per milliliter. Further dilute to obtain a concentration of 30 micrograms per milliliter, using 25 percent dimethyl sulfoxide (one part dimethyl sulfoxide plus three parts distilled water). Use 1 percent potassium phosphate buffer, pH 6.0, and dilute to a concentration of 1 microgram per milliliter. Further dilute this solution with distilled water, and prepare the standard curve containing concentrations of 0.032, 0.04, 0.05, 0.0625, and 0.0781 microgram per milliliter. Add 1.0 milliliter of each concentration to each of six sterile 20 millimeters x 150 millimeters test tubes.

(*c*) *Preparation of sample.* Prepare the sample to be tested, using the same procedure as described for the standard solutions, obtaining six tubes each containing 1.0 milliliter of a solution containing 0.05 microgram per milliliter (estimated).

(*d*) *Preparation of test organism.* The test organism is Candida tropicalis (ATCC 13803), which is maintained on slants of agar described in (*a*) (*1*) of this subdivision. Prepare the daily inoculum by transferring the culture from a stock slant to 100 milliliters of the broth described in (*a*) (*2*) of this subdivision, and incubate for 16 hours to 18 hours at 30° C., with constant shaking. Prepare the daily inoculated assay broth by adding from 3 milliliters to 5 milliliters of a fresh 16 hour to 18 hour broth culture to each liter of assay broth, prepared as directed in (*a*) (*3*) of this subdivision, needed for the test.

(*e*) *Procedure.* To each of the 20 millimeters x 150 millimeters test tubes prepared in (*b*) and (*c*) of this subdivision, add 9.0 milliliters of the inoculated assay broth described in subsection (*d*) of this subdivision, and incubate all standard and sample tubes at 30° C., with constant shaking, for 3 to 5 hours. After incubation, add 0.5 milliliter of a 12 percent formaldehyde solution to each tube, and read the absorbance values in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear uninoculated broth prepared as described in (*a*) (*3*) of this subdivision.

(*f*) *Estimation of potency.* Plot the average values for each concentration of the standard on semilog graph paper with absorbance values on the arithmetic scale and amphotericin concentrations on the logarithmic scale. Construct the best straight line through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L=Calculated absorbance value for the lowest concentration of the standard curve;

H=Calculated absorbance value for the highest concentration of the standard curve;

a, b, c, d, e=Average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straight line. Average the absorbance values for the sample and read the amphotericin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the amphotericin content of the sample.

(2) *Amphotericin A content*—(i) *Amphotericin A.* Accurately weigh about 20 milligrams of the amphotericin A working standard into a 200-milliliter volumetric flask. Add exactly 20.0 milliliters of dimethyl sulfoxide and dissolve. Make to mark with methyl alcohol, and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark, and mix thoroughly.

(ii) *Amphotericin B.* Accurately weigh about 50 milligrams of the amphotericin B working standard into a 50-

milliliter volumetric flask and proceed as directed in subdivision (i) of this subparagraph.

(iii) *Sample.* Accurately weigh about 50 milligrams of the sample to be tested, and proceed as directed in subdivision (i) of this subparagraph. The standards should be used for 1 day only.

(iv) *Blank.* Pipette 5.0 milliliters of dimethyl sulfoxide into a 50-milliliter volumetric flask. Make to mark with methyl alcohol, and mix. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Make to mark with methyl alcohol, and mix thoroughly.

(v) *Procedure.* Use a suitable ultraviolet spectrophotometer and 1-centimeter silica cells. Adjust the instrument to zero with the blank solution. Measure the absorbances of the solutions of standard A, standard B, and the sample at 304 millimicrons and at 282 millimicrons. Calculate the absorptivity of each standard at both wavelengths:

$$\text{Percent amphotericin A} = \frac{[(B \times S_2) - (b \times S_1)] \times 625}{\text{Weight of sample in grams} \times [(B \times a) - (b \times A)]},$$

where:

A = Absorptivity of amphotericin A standard at 282 millimicrons;
B = Absorptivity of amphotericin B standard at 282 millimicrons;
a = Absorptivity of amphotericin A standard at 304 millimicrons;
b = Absorptivity of amphotericin B standard at 304 millimicrons;
S_1 = Absorbance of sample at 282 millimicrons;
S_2 = Absorbance of sample at 304 millimicrons.

(3) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer to each of 5 mice, 0.4 milliliter of a suspension of the drug containing 50 milligrams per milliliter in 0.5 percent gum acacia solution.

(4) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(5) *pH.* Using a 3.0 percent aqueous suspension, proceed as directed in § 141a.5(b) of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(7) *Identity.* Accurately weigh 50.0 milligrams of the sample into a dry 50-milliliter volumetric flask. Add exactly 5 milliliters of dimethyl sulfoxide and dissolve. Make to mark with methyl alcohol, and mix thoroughly. Pipette 4 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to the mark, and mix thoroughly. The sample has absorption peaks at identical wavelengths with that of the standard (272, 282, 295, 362, 381, and 405 millimicrons).

§ 148b.2 Amphotericin B intravenous.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B intravenous is a dry powder containing in each immediate container 50 milligrams of amphotericin B, 41 milligrams of sodium desoxycholate, and suitable buffering substances. It is sterile. It is nontoxic. It is nonpyrogenic. Its moisture content is not more than 8.0 percent. Its pH in an aqueous solution containing 10 milligrams of amphotericin B per milliliter is not less than 7.2 and not more than 8.0. The amphotericin B used conforms to the standards of § 148b.1(a)(1)(i), (ii), (v), (vi), and (vii), except that its amphotericin A content is not more than 5 percent and its residue on ignition is not more than 0.5 percent. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 148.3 of this chapter, each package shall bear on its label and labeling the following statement: "For intravenous infusion in hospitals only." Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency, sterility, toxicity, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph; $5.00 for each container in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $10.00 for all immediate containers in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Dilute the sample in sufficient dimethyl sulfoxide to give a convenient stock solution, and proceed as directed in § 148b.1(b)(1). The amphotericin B content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of amphotericin B that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 141a.2 of this chapter, using the entire contents of each container tested, except that neither penicillinase nor the control tube is used in the test for bacteria and except that the Sabouraud medium is adjusted to pH 7.5–7.9 with sodium hydroxide.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.3 milliliter of an aqueous solution containing 50 micrograms of amphotericin B per milliliter.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter using as a test dose 0.5 milliliter per kilogram of a solution in sterile, pyrogen-free distilled water containing 2 milligrams of amphotericin B per milliliter. If no rabbit shows an individual rise in temperature of 1.1° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 3° C., the sample meets the requirements for absence of pyrogen. If one or two rabbits show a temperature rise of 1.1° C. or more, or if the sum of temperature rises exceeds 3° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show a temperature rise of 1.1° C. or more, and if the sum of the temperature rises does not exceed 8° C. the sample meets the requirements for absence of pyrogens.

(5) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter using an aqueous solution containing 10 milligrams of amphotericin B per milliliter.

PART 148c—COLISTIN

Sec.
148c.1 Colistin sulfate.
148c.2 Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension.
148c.3 Colistin sulfate for oral suspension.
148c.4 Sodium colistimethate.

AUTHORITY: The provisions of this Part 148c issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148c.1 Colistin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate is the white to slightly yellow, odorless sulfate salt of a kind of colistin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 500 micrograms of colistin per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 7.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.0 and not more than 7.0.

(v) It gives a positive identity test for colistin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b). Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4, of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(a) Nutrient agar for the base layer and for preparing the inoculated agar plates as follows:

	Base layer	Seed layer
Pancreatic digest of casein	17.0 gm.	17.0 gm.
Papaic digest of soybean	3.0 gm.	3.0 gm.
Sodium chloride	5.0 gm.	5.0 gm.
Dextrose	2.5 gm.	2.5 gm.
Dibasic potassium phosphate	2.5 gm.	2.5 gm.
Polysorbate 80	------	10.0 ml.
Agar	20.0 gm.	12.0 gm.
Distilled water to make	1,000.0 ml.	1,000.0 ml.
Final pH (after sterilization)	7.2–7.3	7.2–7.3

(b) Make nutrient agar for carrying the organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5–6.6 after sterilization.

In lieu of preparing the medium from the individual ingredients specified, it can be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the specified individual ingredients are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(iii) *Preparation of test organism.* The test organism used is *Bordetella bronchiseptica* (ATCC 4617), which is maintained on slants of agar described in subdivision (ii) (*b*) of this subparagraph. Incubate the slants 24 hours at 37° C. Wash the organism from the slant with 3 milliliters of sterile U.S.P. saline T.S. onto a large agar surface such as provided by a Roux bottle containing 300 milliliters of the agar described in subdivision (ii) (*b*) of this subparagraph. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads and incubate for 24 hours at 37° C. Wash the resulting growth with 50 milliliters of sterile U.S.P. saline T.S. and standardize the organism suspension so that a 1:20 dilution in U.S.P. saline T.S. will give 25 percent light transmission, using a filter having a wavelength of 580 millimicrons and test tube with an inside diameter of 13 millimeters as an absorption cell. Use test plates to determine the quantity (usually 0.3 milliliter) of this standardized bacterial suspension (not the 1:20 dilution) that should be added to each 100 milliliters of melted seed layer agar (cooled to 48° C–50° C.) to give clear, sharp, inhibition zones of appropriate diameter.

(iv) *Preparation of plates.* Use plates the same day they are prepared. Evenly distribute 21.0 milliliters of the melted base layer agar on each Petri dish (20 millimeters x 100 millimeters); cover with porcelain tops, glazed on the outside, and allow the agar to harden. Evenly spread 4.0 milliliters of the inoculated seed layer agar over the surface of the plate. After the seed layer has hardened, place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(v) *Working standard.* Dry an appropriate amount of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and prepare a stock solution of 1,000 micrograms of colistin per milliliter by diluting this dried working standard with 2 milliliters of sterile distilled water and further diluting with 10 percent potassium phosphate buffer, pH 6.0.

(vi) *Standard curve.* Prepare concentrations for the standard curve by diluting the working standard stock solution (1,000 micrograms of colistin per milliliter) with sterile 10 percent potassium phosphate buffer, pH 6.0, to give final concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 1.0 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be 36 one-microgram determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 37° C. and measure the diameter of each zone of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 1.25 microgram per milliliter concentration the average of the 36 readings of the 1.0 microgram per milliliter concentration is 18.0 milliliters, and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 17.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 1.25 microgram per milliliter concentration of those same three plates is 19.6 millimeters, the corrected value is then 19.8 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter for the 36 readings of the 1.0 microgram per milliliter standard;

a, b, d, e = Corrected zone values for the 0.64, 0.80, 1.25, 1.56 microgram per milliliter concentrations respectively.

(vii) *Preparation of sample.* Dissolve an accurately weighed quantity of the sample in sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a convenient stock solution. Further dilute in the same buffer to obtain an estimated concentration of 1.0 microgram per milliliter.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram per milliliter standard and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 37° C. and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, administering a 0.5 milliliter oral test dose of a suspension in sterile distilled water containing 600 micrograms of colistin per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter, using a sample of about 300 milligrams.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity.* Weight about 20 milligrams of sample, add 2 milliliters of 2.5 percent sodium acetate solution and 0.2 milliliter of 0.5 percent triketohydrindene hydrate solution, and bring to boil. A deep-purple color results.

§ 148c.2 Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension.

(a) *Requirements for certification—* (1) *Standards of identity, strength, quality, and purity.* Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension is a suspension containing colistin sulfate, neomycin sulfate, thonzonium bromide, and hydrocortisone acetate, and one or more preservatives, dispersing agents, and buffer substances. Each milliliter contains 3.0 milligrams of colistin, 3.3 milligrams of neomycin, 0.5 milligrams of thonzonium bromide, and 10 milligrams of hydrocortisone acetate. It is sterile. Its pH is not less than 4.8

and not more than 5.2. The colistin sulfate used conforms to the standards prescribed therefor by § 148c.1(a)(1), except for toxicity. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (v), (vi), and (vii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 141.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for colistin content, neomycin content, sterility, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For Sterility: 10 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii)(*c*)(*1*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*c*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Colistin content.* Proceed as directed in § 148c.1(b)(1), except prepare the sample as follows: Thoroughly mix the sample and transfer an accurately measured aliquot of the sample into a 100-milliliter volumetric flask. Fill the flask to mark with 10 percent potassium phosphate buffer, pH 6.0. Further dilute to the proper estimated concentration in 10 percent potassium phosphate buffer, pH 6.0. Its content of colistin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of colistin per milliliter that it is represented to contain.

(2) *Neomycin content.* Proceed as directed in § 148i.1(b)(1) of this chapter, except prepare the sample as follows: Thoroughly mix the sample and transfer an accurately measured aliquot to a 100-milliliter volumetric flask. Fill to mark with 0.1*M* potassium phosphate buffer, pH 8.0 Further dilute to the proper estimated concentration in pH 8.0 buffer. Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin per milliliter that it is represented to contain.

(3) *Sterility.* Use 0.25 milliliter from each container tested and proceed as directed in § 141a.2 of this chapter except that neither penicillinase nor the control tube is used in the test for bacteria.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution or suspension.

§ 148c.3 Colistin sulfate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate for oral suspension is a dry mixture of colistin sulfate, with or without one or more suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, and flavorings. The colistin sulfate content is 5.0 milligrams of colistin per milliliter of the reconstituted suspension. The moisture content is not more than 3.0 percent. The pH of the reconstituted suspension is not less than 5.0 and not more than 6.0. The colistin sulfate used conforms to the standards prescribed by § 148c.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled as prescribed in § 148.3, of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than approximately 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148c.1(b)(1), except prepare the sample as follows: Reconstitute the suspension as directed in the label. Remove a single-dose aliquot of the reconstituted suspension with a syringe and dilute in 1.0 percent potassium phosphate buffer, pH 6.0. The colistin content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of colistin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5 of this chapter, using the suspension when reconstituted as directed in the labeling.

§ 148c.4 Sodium colistimethate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium colistimethate is the sodium salt of a kind of colistin methane sulfonate or a mixture of two or more such salts. It is a white to slightly yellow, odorless, fine powder which is freely soluble in water. It is so purified and dried that:

(i) Its potency is not less than 300 micrograms of colistin base per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 9.0 percent.

(vii) Its pH in an aqueous solution contains 10 milligrams per milliliter is not less than 6.5 and not more than 9.0.

(viii) It gives a positive identity test for sodium colistimethate.

(ix) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, identity, and heavy metals.

(ii) Samples required:

(*a*) For all tests except sterility: 10 containers, each containing not less than 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $5.00 for each container in the sample submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148c.1(b)(1) except use the sodium colistimethate working standard the same day it is prepared.

(2) *Sterility.* Use the entire contents of a container or the equivalent of approximately 500 milligrams of colistin from each large container and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1.0 milliliter per kilogram of a solution in sterile, pyrogen-free distilled water, containing 10 milligrams of colistin per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution in

sterile distilled water containing 1.5 milligrams of colistin per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using a test dose of 1 milliliter per kilogram of a solution containing 3.0 milligrams of colistin per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter, using a sample of not less than 100 milligrams.

(7) *pH.* Proceed as directed in § 141a.5 (b) of this chapter, using a 1 percent aqueous solution prepared in the following manner: Weigh accurately 0.5 gram of sample and transfer to a 125-milliliter Erlenmeyer flask. Add 50 milliliters of freshly boiled distilled water, stopper, and shake until the sample is in solution. Allow to stand for one-half hour before determining the pH.

(8) *Identity.* To about 20 milligrams of sample add 2 milliliters of 2.5 percent sodium acetate solution and 0.2 milliliter of 0.5 percent triketohydrindene hydrate solution. Bring to boil. No pink color results. To about 20 milligrams of sample add 2 milliliters of dilute hydrochloric acid. Reflux for about 2 minutes, neutralize with 10 percent sodium hydroxide, and add sodium acetate. A deep-purple color results.

(9) *Heavy metals.* Proceed as directed in § 148m.1(b)(9) of this chapter.

PART 148d—CYCLOSERINE

Sec.
148d.1 Cycloserine.
148d.2 Cycloserine capsules.
148d.3 Cycloserine capsules with isoniazid.

AUTHORITY: The provisions of this Part 148d issued under sec. 507, 59 Stat. 468, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148d.1 Cycloserine.

(a) *Requirements for certification — (1) Standards of identity, strength, quality, and purity.* Cycloserine is a white to slightly yellowish crystalline compound. It has the chemical structure D-4-amino-3-isoxazolidone. It is so purified that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in a 10 percent aqueous solution is not less than 5.5 and not more than 6.5.

(v) Its residue on ignition is not more than 0.5 percent.

(vi) It gives a positive identity for cycloserine.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, residue on ignition, crystallinity, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—(1) Potency—(i) Colorimetric assay—(a) Stock standard solution.* Dry approximately 100 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient 0.1*N* sodium hydroxide to give a solution containing 1,000 micrograms per milliliter.

(*b*) *Standard curve solutions.* Pipette accurately 0.0, 1.0, 5.0, 10.0, 15.0, and 20.0 milliliters of the stock standard solution to each of six 100-milliliter volumetric flasks, dilute to 100 milliliters with 0.1*N* sodium hydroxide and mix thoroughly.

(*c*) Reagents:

(*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of 4 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in an amber bottle.

(*d*) *Procedure.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask. Dissolve in sufficient 0.1*N* sodium hydroxide to measure exactly 100 milliliters. Mix thoroughly and transfer 10 milliliters to a second 100-milliliter volumetric flask, and mix thoroughly. Transfer exactly 1.0 milliliter of each of the standard curve solutions and of the sample solution to respective test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid to each of the test tubes. Mix thoroughly. Add exactly 1.0 milliliter of oxidized nitroprusside reagent to each test tube and mix thoroughly. Allow the tubes to stand at room temperature for at least 10 minutes in order that maximum color intensity may develop. Using the solution containing 0.0 milliliter of working standard as a blank, determine the absorbances of the solutions at 625 millimicrons in a suitable spectrophotometer. Plot concentration versus absorbance on linear graph paper. The curve may deviate slightly from a straight line. The standard curve solutions equal 0, 10, 50, 100, 150, and 200 micrograms of cycloserine, respectively.

(*e*) Calculations:

$$\text{Micrograms cycloserine per milligram} = \frac{\text{Concentration in micrograms from calibration curve} \times 1{,}000}{\text{Weight of original sample in milligrams}}.$$

(ii) *Plate assay—(a) Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*1*) Base layer agar:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) Seed layer agar (also used for maintaining test organism):

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

In lieu of preparing the medium from the individual ingredients specified, it can be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the individual ingredients specified in subdivision (ii)(*b*) (*1*) and (*2*) of this subparagraph are permissible if the resulting media possess growth-promoting properties equal to the media described.

(*c*) *Preparation of test organism.* The test organism used is *Staphylococcus aureus* (ATCC 6538P). Incubate a seed agar slant that has been streaked with the organism, for 24 hours at 37° C. Wash the slant with 3 milliliters of sterile U.S.P. saline T.S. with the aid of glass beads onto a Roux bottle containing 300 milliliters of seed agar. Incubate for 24 hours at 37° C. and wash with 50 milliliters of sterile U.S.P. saline T.S. into an Erlenmeyer flask. Using a suitable photoelectric colorimeter with a filter having a wavelength of 580 millimicrons, and with a test tube having an inside diameter of 13 millimeters, as an absorption cell, adjust the suspension so that a 1:20 dilution with U.S.P. saline T.S. will give 25 percent light transmission. Determine by test plates the amount of inoculum to be added to the seed layer to obtain satisfactory zones (usually 2 percent of a 1:50 dilution).

(*d*) *Preparation of plates.* Use plates the same day they are prepared. Evenly distribute 10.0 milliliters of the melted base layer agar on each Petri dish (20 millimeters x 100 millimeters); cover with porcelain tops, glazed on the outside, and allow the agar to harden. Evenly spread 4.0 milliliters of the inoculated seed layer agar over the surface of the plate. After the seed layer has hardened, place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*e*) *Standard.* Dry an appropriate amount of the working standard for 3 hours at 60° C. with a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient distilled water to obtain a stock solution of 1,000 micrograms per

milliliter. This solution may be used for 2 days if kept under refrigeration.

(*f*) *Standard curve.* Prepare concentrations for the standard curve by diluting the working standard stock solution (1,000 micrograms per milliliter) in 1 percent potassium phosphate buffer, pH 6.0, to give final concentrations of 32, 40, 50, 62.5, and 78.1 micrograms per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 50 micrograms per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with 50 micrograms per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be thirty-six 50-microgram determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 30° C., and measure the diameter of each zone of inhibition. Average the readings of the 50 micrograms per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 50 micrograms per milliliter concentration. The average of the 36 readings of the 50 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 50 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 62.5 micrograms per milliliter concentration the average of the 36 readings of the 50 micrograms per milliliter concentration is 18.0 millimeters and the average of the 50 micrograms per milliliter concentration of this set of three plates is 17.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 62.5 micrograms per milliliter concentration of those same three plates is 19.6 millimeters, the corrected value is then 19.8 millimeters. Plot these corrected values, including the average of the 50 micrograms per milliliter concentration, on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L=Calculated zone diameter for the lowest concentration of the standard;

H=Calculated zone diameter for the highest concentration of the standard;

c=Average zone diameter for the 50 micrograms per milliliter standard;

a, b, d, e=Corrected average value for the 32, 40, 62.5, and 78.1 microgram per milliliter standard solution, respectively.

(*g*) *Preparation of Sample.* Dissolve an accurately weighed quantity of the sample in 1 percent potassium phosphate buffer, pH 6.0, to an estimated concentration of 50 micrograms per milliliter.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 50 micrograms per milliliter standard and three cylinders with the 50 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 30° C. and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 50 micrograms per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 50 micrograms per milliliter value on the curve. From the curves, read the potencies corresponding to these corrected values of zone sizes.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution in sterile U.S.P. saline T.S.

NOTE: Solution must be administered within ½-hour after preparation.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution with a concentration of 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(7) *Identity.* Proceed as directed in subparagraph (1)(i) of this paragraph.

§ 148d.2 Cycloserine capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cycloserine capsules are capsules composed of crystalline cycloserine, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each capsule contains 250 milligrams of cycloserine. The moisture content is not more than 1.0 percent. The cycloserine used conforms to the standards prescribed by § 148d.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3 of this chapter, the labeling of each package shall bear a warning to the effect that the drug is to be used in patients with tuberculosis who fail to respond to treatment with isoniazid, streptomycin, para-aminosalicylic acid, viomycin, pyrazinamide, or combinations of these drugs, and that the drug may cause serious reactions such as convulsive seizures and mental disturbances. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Cycloserine used in making the batch for potency, toxicity, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for cycloserine content and moisture.

(ii) Samples required:

(*a*) Cycloserine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $.75 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Chemical colorimetric assay.* (*a*) Reagents:

(*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of the 4.0 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in amber bottle.

(*5*) Cycloserine standard solution—Prepare a standard solution of the cycloserine working standard in 0.1*N* sodium hydroxide to contain 100 micrograms per milliliter.

(*b*) *Procedure.* Transfer the contents of 10 capsules into a 1,000-milliliter volumetric flask. Add 0.1*N* sodium hydroxide to dissolve the sample, and add sufficient 0.1*N* sodium hydroxide to measure 1,000 milliliters. Mix well, and filter. Dilute an aliquot of the filtrate with sufficient 0.1*N* sodium hydroxide to give a concentration of 0.1 milligram per milliliter (estimated) and mix well. Pipette exactly 1.0 milliliter of the working standard solution and 1.0 milliliter of the sample solution into separate test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid and exactly 1.0 milliliter of oxidized nitroprusside reagent to each of the test tubes; then mix thoroughly. Allow the tubes to stand at room temperature for 10 minutes–15 minutes, in order that maximum color intensity may develop. Using a reagent blank, determine the absorbance of the solutions at 625 millimicrons in a suitable spectrophotometer.

Calculation:

$$\text{Milligrams of cycloserine per capsule}=\frac{\text{Sample absorbance}}{\text{Standard absorbance}}\times\text{Labeled potency per capsule in milligrams.}$$

(ii) *Biological assay.* Using a high-speed blender, prepare an appropriate number of capsules in 500 milliliters of 1 percent potassium phosphate buffer, pH 6.0, and proceed as directed in § 148d.1(b)(1)(ii).

The average potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of cycloserine that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148d.3 Cycloserine capsules with isoniazid.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Cycloserine capsules with isoniazid are capsules composed of crystalline cycloserine and isoniazid, with or without, one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each capsule contains 250 milligrams of cycloserine and 150 milligrams of isoniazid. The moisture content is not more than 1.0 percent. The cycloserine used conforms to the standards prescribed by § 148d.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3 of this chapter, the labeling of each package shall bear a warning to the effect that the drug is to be used in patients with tuberculosis who fail to respond to treatment with isoniazid, streptomycin, paraaminosalicyclic acid, viomycin, pyrazinamide, or combinations of these drugs and that the drug may cause serious reactions such as convulsive seizures and mental disturbances. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.3 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Cycloserine used in making the batch for potency, toxicity, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for cycloserine content, isoniazid content, and moisture.

(ii) Samples required:

(*a*) Cycloserine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the sample submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph; $1.00 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay—(1) Potency—(i) Chemical assays for cycloserine and isoniazid—(a) Preparation of sample.* Transfer the entire contents of four representative capsules to a 1,000-milliliter volumetric flask. Dilute to volume with distilled water, and mix well. Filter about 30 milliliters of the solution, discarding the first few milliliters of filtrate. Collect the remaining filtrate. Transfer a 10.0-milliliter aliquot of the filtrate to a 1,000-milliliter volumetric flask, dilute to volume with 0.1*N* hydrochloric acid, and mix well.

(*b*) *Preparation of cycloserine standard.* Accurately weigh about 30 milligrams of the cycloserine working standard and dissolve in sufficient distilled water to give a concentration of 1.0 milligram per milliliter. Transfer a 10.0-milliliter aliquot of this solution to a 1,000-milliliter volumetric flask. Dilute to volume with 0.1*N* hydrochloric acid, and mix well.

(*c*) *Preparation of isoniazid standard.* Accurately weigh about 30 milligrams of pure isoniazid and dissolve in sufficient distilled water to give a concentration of 1.0 milligram per milliliter. Transfer a 10.0-milliliter aliquot of this solution to a 1,000-milliliter volumetric flask. Dilute to volume with 0.1*N* hydrochloric acid, and mix well.

(*d*) *Procedure.* Adjust a suitable ultraviolet spectrophotometer to 100 percent light transmission with 0.1*N* hydrochloric acid in the absorption cell. Determine the absorbance values for the sample, the cycloserine standard, and the isoniazid standard at the absorption peaks at 219 millimicrons and 268 millimicrons. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 210 millimicrons to 290 millimicrons. If a nonrecording spectrophotometer is used, determine the absorbance values at the absorption peaks. (The exact position of the peaks should be determined for the particular instrument used.)

(*e*) *Calculations*—(*1*) Cycloserine content: Determine the ratio (*R*) of the absorbance of the isoniazid standard at 219 millimicrons to its absorbance at 268 millimicrons.

$$R=\frac{\text{Absorbance of isoniazid standard at 219 millimicrons}}{\text{Absorbance of isoniazid standard at 268 millimicrons}}$$

Correct the absorbance value of the sample at 219 millimicrons for the isoniazid equivalent as follows:

$$A_c=(A_1-A_2\times R),$$

where:

A_c=Corrected absorbance at 219 millimicrons;

A_1=Absorbance of sample at 219 millimicrons;

A_2=Absorbance of sample at 268 millimicrons.

Calculate the cycloserine content as follows:

$$\text{Milligrams of cycloserine per capsule}=\frac{A_c}{A_s}\times 250,$$

where:

A_s=Absorbance of cycloserine standard at 219 millimicrons.

The content of cycloserine is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of cycloserine that it is represented to contain.

(2) *Isoniazid content—(i) Preparation of sample.* Use the solution prepared as directed in subdivision (i)(*a*) of this subparagraph.

(*ii*) *Preparation of standard.* Use the solution prepared as directed in subdivision (i)(*c*) of this subparagraph.

(*iii*) *Procedure.* Proceed as directed in subdivision (i)(*d*) of this subparagraph and obtain the absorbance of the sample and the isoniazid standard at the absorption peak at 268 millimicrons. Calculate as follows:

$$\text{Milligrams of isoniazid per capsule}=\frac{A_2\times 250}{A_1},$$

where:

A_1=Absorbance of isoniazid standard at 268 millimicrons;

A_2=Absorbance of sample at 268 millimicrons.

The content of isoniazid is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Plate assay method for cycloserine content.* Proceed as directed in § 148d.2(b)(1)(ii). The average content of cycloserine in each capsule is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams that each capsule is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

PART 148f—GRAMICIDIN

§ 148f.1 Gramicidin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Gramicidin is the white, or nearly white, odorless, crystalline compound of a kind of gramicidin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It has a potency of not less than 900 micrograms of gramicidin per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 3 percent.

(iv) Its residue on ignition is not more than 1.0 percent.

(v) It does not melt below 229° C. after drying in vacuum at 60° C. for 3 hours.

(vi) The difference between the absorptivity value at the maximum occurring at 282 millimicrons and the absorptivity value at the minimum occurring at 247 millimicrons is 100±4 percent of the difference obtained with the gramicidin working standard.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, residue on ignition, melting point, identity, and crystallinity.

(ii) Samples required of the batch: Ten packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make nutrient broth for preparing an inoculum of the test organism, as follows:

Peptone	5.0 gm.
Yeast extract	1.5 gm.
Beef extract	1.5 gm.
Sodium chloride	3.5 gm.
Dextrose	1.0 gm.
Dipotassium phosphate	3.68 gm.
Potassium dihydrogen phosphate	1.32 gm.
Distilled water, q.s.	1,000.00 ml.

pH 7.0 after sterilization.

In lieu of preparing the medium from the individual ingredients specified above, the nutrient broth may be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the individual ingredients specified are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(ii) *Working standard.* Dry an appropriate amount of the gramicidin working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve in sufficient 95 percent alcohol to obtain stock solution of 1,000 micrograms per milliliter. Keep this stock solution in the refrigerator. Do not use it later than 30 days after it is made.

(iii) *Preparation of sample.* Dissolve an appropriate quantity of the sample in a volume of 95 percent ethyl alcohol to obtain a convenient stock solution. Make further dilutions volumetrically in 95 percent ethyl alcohol to obtain a final concentration of 0.04 microgram per milliliter (estimated).

(iv) *Preparation of test organism.* The test organism is Streptococcus faecalis (ATCC 10541) which is maintained in 100-milliliter quantities of the nutrient broth described in subdivision (i) of this subparagraph. For the test, prepare a fresh subculture by transferring a loopful of the stock culture to 100 milliliters of nutrient broth, and incubate 16 hours to 18 hours at 37° C. For the daily inoculum, use 6.0 milliliters of the subculture for each 1,000 milliliters of nutrient broth needed in the assay.

(v) *Procedure.* Prepare solutions for the daily standard curve by diluting an aliquot of the stock working standard solution prepared as described in subdivision (ii) of this subparagraph in 95 percent ethyl alcohol to obtain concentrations of 0.028, 0.034, 0.040, 0.048, and 0.058 microgram per milliliter. Place 0.1 milliliter of each concentration of the standard curve and of the sample solution prepared as described in subdivision (iii) of this subparagraph in each of three replicate test tubes, 16 millimeters x 125 millimeters (outside dimensions). To each tube, add 9 milliliters of the inoculated broth described in subdivision (iv) of this subparagraph and place immediately in a water bath at 37° C. for 3 hours to 4 hours. Remove the tubes and add 0.5 milliliter of a 12 percent solution of formaldehyde to each tube. Read the absorbance values of each tube in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear, uninoculated broth prepared as described in subdivision (i) of this subparagraph.

(vi) *Estimation of potency.* Plot the average absorbance values for each concentration of the standard on two-cycle semi-logarithmic graph paper with absorbance values on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight line through the points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated absorbance value for the lowest concentration of the curve;

H = Calculated absorbance value for the highest concentration of the standard curve;

a, b, c, d, e = Average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straight line. Average the absorbance value for the sample and read the gramicidin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the gramicidin content of the sample.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except inject subcutaneously with 0.5 milliliter of a suspension of the sample containing 5 milligrams of gramicidin per milliliter in 10 percent acacia solution. Observe mice for 4 days.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *Residue on ignition.* Proceed as directed in § 141c.401(g) of this chapter.

(5) *Melting point.* Proceed as directed in § 141d.301(h) of this chapter.

(6) *Identity.* Accurately weigh about 20 milligrams of the sample and dilute in ethyl alcohol to give a concentration of 0.02 milligram (estimated) of gramicidin per milliliter. Prepare a solution of the gramicidin working standard to contain 0.02 milligram per milliliter in ethyl alcohol. Using a suitable recording spectrophotometer with 1-centimeter cells, record the ultraviolet absorbance spectrum of each solution from 220 millimicrons to 320 millimicrons. The ultraviolet absorbance spectrum of the sample solution should compare qualitatively to that of the working standard solution. Determine the absorptivities of each at the maximum occurring at 282 millimicrons and at the minimum occurring at 247 millimicrons (the exact position of the maximum and minimum of the gramicidin working standard should be determined for the particular instrument used). The difference in the absorptivities of the sample is 100±4.0 percent of that of the working standard.

(7) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(Sec. 507, 59 Stat. 463, as amended, 76 Stat. 785, 786, 787; 21 U.S.C. 357)

PART 148h—KANAMYCIN SULFATE

Sec.

148h.1 Kanamycin sulfate.
148h.2 Kanamycin sulfate injection.
148h.3 Kanamycin sulfate capsules.

AUTHORITY: The provisions of this Part 148h issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148h.1 Kanamycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate is the crystalline sulfate salt of a kind of kanamycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency on the anhydrous basis is not less than 750 micrograms of kanamycin per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 4 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not more than 1.0 percent.

(viii) It gives a positive identity test for kanamycin.

(ix) It contains not more than 5.0 percent kanamycin B.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, residue on ignition, identity, crystallinity, and kanamycin B content.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $5.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described under § 141a.1(a) of this chapter.

(ii) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make nutrient agar as follows:

(*a*) For the base and seed layers:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.

Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000 ml.

pH 7.8 to 8.0 after sterilization.

(*b*) Nutrient agar for carrying the test organism is the same as that described in (*a*) of this subdivision, except that the pH is 6.5 to 6.6 after sterilization.

In lieu of preparing the media from the individual ingredients specified in this subdivision, they may be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Working standard.* Dissolve a suitable quantity of the working standard, accurately weighed, in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. The stock solution, when stored under refrigeration, may be used for a period not exceeding 1 month.

(iv) *Preparation of sample.* Dissolve a suitable quantity, accurately weighed, in 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. Further dilute volumetrically in 0.1*M* potassium phosphate buffer, pH 8.0, to make a solution containing 5.0 micrograms of kanamycin per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is *Staphylococcus aureus* (ATCC 6538P) which is maintained on agar described in subdivision (ii) (*b*) of this subparagraph. From a stock slant, inoculate a Roux bottle containing the same agar and incubate for 24 hours at 32° C. to 35° C. Wash the growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S. If an aliquot of this bulk suspension, when diluted 1:20 with sterile U.S.P. saline T.S., gives 25 percent light transmission in a suitable photoelectric colorimeter equipped with a filter having a wavelength of 580 $m\mu$ and a test tube with an inside diameter of 13 millimeters as an absorption cell, it is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution, so that an aliquot of the adjusted suspension diluted 1:20 gives 25 percent light transmission. (The adjusted bulk suspension only, and not the 1:20 dilution of it, is used in preparing the seed layer.) The bulk suspension may be stored for 1 week under refrigeration. An aliquot of the adjusted bulk suspension is added to the agar described in subdivision (ii) (*a*) of this subparagraph, which has been melted and cooled to 48° C. Employ test plates to determine the quantity of the adjusted bulk suspension (usually 0.4 milliliter) to be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of an appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) (*a*) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow to harden. Use the plates the same day they are prepared. Add 4.0 milliliters of the inoculum prepared as described in subdivision (v) of this subparagraph to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface.

(vii) *Standard curve.* Prepare daily in 0.1*M* potassium phosphate buffer, pH 8.0, from the stock solution described in subdivision (iii) of this subparagraph, concentrations of 3.2, 4.0, 5.0, 6.25, and 7.81 micrograms per milliliter of solution. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution except the 5.0 micrograms per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates, fill three cylinders with the 5.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 5-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 5.0 micrograms per milliliter concentration and the readings of the concentration tested for each set of three plates and average also all 36 readings of the 5.0 micrograms per milliliter concentration. The average of the 36 readings of the 5.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 5.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 4.0-microgram concentration, the average of the 36 readings of the 5.0 micrograms per milliliter concentration is 16.5 millimeters and the average of the 5.0 micrograms per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 4.0 micrograms per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 5.0 micrograms per milliliter standard;

a, b, d, e = Corrected average values for the 3.2, 4.0, 6.25, and 7.81 micrograms per milliliter solutions, respectively.

Plot the values obtained for L and H and connect wtih a straight line.

(viii) *Assay.* Place six cylinders on the inoculated agar surface in each Petri dish prepared as described in subdivision (vi) of this subparagraph, so that they are at approximately 60° intervals on 2.8-centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 5.0 micrograms per milliliter standard and three cylinders with the 5.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition.

(ix) *Estimation of potency.* Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives larger average zone size than the average of the standard, add the difference between them to the 5.0-microgram zone sizes of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 5.0-microgram value on the curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using 0.5 milliliter of a solution prepared by diluting the sample to approximately 2.0 milligrams of kanamycin per milliliter with sterile U.S.P. saline T.S.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1.0 milliliter per kilogram of solution containing 10 milligrams of kanamycin per milliliter of sterile, pyrogen-free U.S.P. saline T.S.

(5) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 10 milligrams per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(8) *Identity.* Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water, and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol, then add 0.5 milliliter of pyridine. Heat on a steam bath for 5 minutes, and add 10 milliliters of water; a deep-purple color is produced.

(9) *Kanamycin B content*—(i) *Cylinders (cups).* Use cylinders described under § 141a.1(a) of this chapter.

(ii) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make agar for the base and seed layers as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.

ph 7.8 to 8.0 after sterilization.

(iii) *Working standard.* Dissolve a suitable quantity of the kanamycin sulfate reference standard, accurately weighed, in 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration

equivalent to 1.0 milligram of kanamycin per milliliter.

(iv) *Preparation of sample.* To 50 milligrams, accurately weighed, of kanamycin sulfate in a suitable container (such as a 7.5-milliliter serum vial) add 2.5 milliliters of 6*N* hydrochloric acid, and tightly close the container. Heat in a water bath at 100° C. for 1 hour, and cool. Add 2 milliliters of 6*N* sodium hydroxide, then dilute with sterile 0.1*M* potassium phosphate buffer, pH 8.0, to obtain a concentration of the equivalent of 1 microgram of kanamycin B per milliliter (estimated).

(v) *Preparation of test organism.* Use Bacillus subtilis (ATCC 6633) maintained on the nutrient agar described in subdivision (ii) (*b*) of this subparagraph. From a stock slant, inoculate a Roux bottle containing approximately 300 milliliters of the same agar and incubate for 24 hours at 32° C. to 35° C. Prepare a spore suspension by the following method: Grow the organism for 5 days in a Roux bottle containing 300 milliliters of nutrient agar described in (ii) (*b*) of this subparagraph, modified by the addition of 300 milligrams of $MnSO_4 \cdot H_2O$ per liter of nutrient agar. Wash and heat-shock the spore suspension as described in (v) (*a*) of this subparagraph. Maintain the spore suspension under refrigeration. Determine by appropriate tests the quantity of spore suspension to be added to each 100 milliliters of agar for the secondary layer that will give clear, sharp zones of inhibition.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow to harden. Use the plates the same day they are prepared. Add 4.0 milliliters of the fresh daily inoculum described in subdivision (iv) of this subparagraph to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface.

(vii) *Standard curve.* Prepare on the day of testing in 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, from the standard stock solution, sufficient volumes of the following concentrations: 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms per milliliter. The 1.0 microgram per milliliter solution is the reference point of the standard curve. On each of three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1.0-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the concentration test for each set of three plates and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram per milliliter concentration is 16.5 millimeters and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilogarithmic paper, using the concentration in micrograms per milliliter as the ordinate and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L = \frac{3a+2b+c-e}{5},$$

$$H = \frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 1.0 microgram per milliliter standard;

a, b, d, e = Corrected average values for the 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms per milliliter solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Place six cylinders on the inoculated agar surface in each Petri dish prepared as described in subdivision (vi) of this subparagraph, so that they are at approximately 60° intervals on a 2.8-centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram per milliliter standard and three cylinders with the 1.0 microgram (estimated) per milliliter sample, alternating standard and sample. Incubate plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition.

(ix) *Estimation of kanamycin B content.* Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives larger average zone size than the average of the standard, add the difference between them to the 1.0-microgram zone sizes of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0-microgram value on the curve. From the curve, read the kanamycin potencies corresponding to these corrected values of zone sizes. Multiply the observed potency by 100 and divide by 126, to obtain a value representing the potency in terms of the milligram equivalent of kanamycin B. The calculated amount of kanamycin B is not more than 5 percent of the content of kanamycin found in subparagraph (1) (i) of this paragraph.

(10) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148h.2 Kanamycin sulfate injection.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate injection is an aqueous solution of kanamycin sulfate with suitable and harmless buffer substances and preservatives. It contains either 250 milligrams of kanamycin per milliliter or 1.0 gram of kanamycin per 3.0 milliliters. It is sterile. It is nontoxic. It is nonpyrogenic. Its pH is not less than 3.5 and not more than 5.0. The kanamycin sulfate used conforms to the standards prescribed by § 148h.1(a) (1) (i), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* (i) In addition to the requirements prescribed by § 148.3 of this chapter, the labeling of each package shall bear a warning to the effect that older patients and patients receiving a total dose of more than 20 grams of the drug should be carefully observed for signs of eighth-nerve damage. In patients with impaired kidney function or with prerenal azotemia, the risk of severe ototoxic reaction that may result in permanent deafness is sharply increased.

(ii) The expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, residue on ignition, moisture, identity, crystallinity, and kanamycin B content.

(*b*) The batch for potency, sterility, toxicity, pyrogens, histamine, and pH.

(ii) Samples required:

(*a*) The kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: Minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each container in the samples submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph; $5.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3) (ii) (*b*) (*2*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148h.1(b) (1), except prepare the sample for assay by introducing a representative aliquot of the solution equivalent to one dose into a 100-milliliter volumetric flask and dilute to mark with 0.1*M* potassium phosphate buffer, pH 8.0. Mix thor-

oughly and make to proper estimated dilutions in pH 8.0 buffer to the prescribed reference point. The average potency is satisfactory if the immediate containers contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Sterility.* Using the entire contents of single-dose containers or the equivalent of approximately 500 milligrams from each multiple-dose container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 148h.1(b)(3).

(4) *Pyrogens.* Proceed as directed in § 148h.1(b)(4).

(5) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using undiluted solution.

§ 148h.3 Kanamycin sulfate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate capsules are composed of crystalline kanamycin sulfate, with or without one or more suitable and harmless buffer substances, vegetable oils, preservatives, diluents, binders, lubricants, colorings, and flavorings, enclosed in gelatin capsules. Each capsule contains 500 milligrams of kanamycin. The moisture content is not more than 4.0 percent. The crystalline kanamycin sulfate used conforms to the standards prescribed by § 148h.1(a)(1) (i), (iii), (v), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, toxicity, moisture, pH, residue on ignition, identity, and kanamycin B content.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148h.1(b)(1), except prepare the sample for assay as follows: Place a representative number of capsules in a glass blending jar containing 500 milliliters of 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0. Using a high-speed blender, blend 3 minutes to 5 minutes, and then remove an aliquot and make to the proper estimated dilution in pH 8.0 buffer.

The average potency of the capsules is satisfactory if they contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

PART 148m—OLEANDOMYCIN

§ 148m.1 Oleandomycin phosphate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oleandomycin phosphate is the crystalline phosphate salt of a kind of oleandomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 775 micrograms of oleandomycin per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 5 percent.

(vi) Its pH in a solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 6.0.

(vii) Its residue on ignition is not more than 1 percent.

(viii) Its heavy metals content is not more than 100 parts per million.

(ix) It gives a positive identity test.

(2) *Packaging.* In addition to the requirements of § 148.2 of this chapter, if it is packaged for dispensing and is intended for intravenous or intramuscular use it shall contain 500 milligrams of oleandomycin.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(4) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays of the batch for potency, sterility, pyrogens, toxicity, moisture, pH, crystallinity, residue on ignition, heavy metals, and identity.

(ii) Samples of the batch:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing equal portions of not less than 500 milligrams.

(*2*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers of the batch.

(*2*) For sterility: 10 immediate containers of the batch.

(5) *Fees.* $5.00 for each container submitted in accordance with subparagraph (4)(ii)(*a*)(*1*) and (*b*)(*1*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (4)(ii)(*a*)(*2*) and (*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media*—(*a*) Make nutrient agar for the seed and base layers as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.8 to 8.0 after sterilization.

(*b*) Use the nutrient agar described in subsection (*a*) of this subdivision for maintaining the test organism, except that its pH after sterilization is 6.5 to 6.6. In lieu of preparing media from the individual ingredients specified in this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the individual ingredients specified in this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Working standard.* Dissolve a suitable weighed quantity (usually 25 milligrams or less) of the working standard in 2 milliliters of ethyl alcohol, then add sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration of 1,000 micrograms of oleandomycin base per milliliter. This stock solution may be kept in the refrigerator for 30 days.

(iv) *Preparation of sample.* Dissolve the sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 5.0 micrograms of oleandomycin per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is Staphylococcus epidermidis (ATCC 12228), which is maintained on slants of agar described in subdivision (ii)(*b*) of this subparagraph. Wash the organism from the agar slant with 3 milliliters of sterile U.S.P. saline T.S. onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in subdivision (ii)(*b*) of this subparagraph. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C. to 35° C. and then wash the resulting growth from the agar surface with about 50 milliliters of sterile U.S.P. saline T.S. Standardize the suspension so that a 1:25 dilution of it will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the bulk suspension (usually 0.16 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) (*a*) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in subdivision (ii) (*a*) of this subparagraph, cool to 48° C., add the proper amount of the test organism as described in subdivision (v) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1*M* potassium phosphate buffer, pH 8.0, to obtain concentrations of 3.2, 4.0, 5.0, 6.25 and 7.80 micrograms of oleandomycin per milliliter. Use three plates for the determination of each point on the curve, except the 5.0 micrograms per milliliter concentration, a total of 12 plates. On each of three plates, fill three cylinders with the 5.0 micrograms per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 5-microgram determinations and nine determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 5.0 micrograms per milliliter concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the 5.0 micrograms per milliliter concentration. The average of the 36 readings of the 5.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 5.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 4.0-microgram concentration, the average of the 36 readings of the 5.0 microgram concentration were 20 millimeters and the average of the 5.0-microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 4.0-microgram concentration of these same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentrations in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 5.0 micrograms per milliliter standard;

a, b, d, e = Corrected average values for 3.2 4.0, 6.25, and 7.81 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 5.0 micrograms per milliliter solution and three cylinders with the 5.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 5.0 micrograms per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 5.0 micrograms per milliliter value on the curve, and read the potencies corresponding to these corrected values of zone sizes.

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using sterile, pyrogen-free saline T.S. as a diluent, and inject 1.0 milliliter per kilogram of a solution containing 10 milligrams of oleandomycin per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except inject 0.5 milliliter of a solution in sterile saline T.S. containing 8 milligrams of oleandomycin per milliliter.

(5) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, except use a sample of approximately 1 gram, accurately weighed.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 100 milligrams of the sample per milliliter.

(7) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(8) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(9) *Heavy metals*—(i) *Preparation of reagents*—(*a*) *Ammonia, T.S.* Prepare an aqueous solution containing not less than 9 grams and not more than 10 grams of NH_3 per 100 milliliters.

(*b*) *Diluted acetic acid.* Dilute 60.0 milliliters of glacial acetic acid with sufficient water to give a solution of 1,000 milliliters.

(*c*) *Hydrogen sulfide, T.S.* Prepare a saturated solution of hydrogen sulfide by passing H_2S into cold water for a sufficient time. It is suitable if it produces at once a copious precipitate when added to an equal volume of 1*N* ferric chloride. Prepare a fresh solution of hydrogen sulfide T.S. each time a metals test is performed.

(*d*) *Lead nitrate stock solution.* Dissolve 159.8 milligrams of lead nitrate with 100 milliliters of water to which has been added 1 milliliter of nitric acid, and adjust with water to a volume of 1,000 milliliters. Prepare and store this solution in glass containers free from soluble lead salts.

(*e*) *Standard lead solution.* Dilute a 10.0-milliliter aliquot of the lead nitrate stock solution to 100 milliliters with water. This solution must be freshly prepared each time a heavy metals test is performed. One milliliter of this standard lead solution represents a level of 10 parts per million of lead.

(ii) *Preparation of the sample.* Use the sulfated ash obtained in the ash content test, or if necessary ash a separate weighing of 1.0 gram, accurately weighed, or 0.5 gram if the heavy metals limit is more than 30 parts per million. If a separate weighing of the substance is made, carefully ignite in a porcelain crucible at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain lid during the charring. Add to the contents of the crucible 2 milliliters of nitric acid and 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace at 500° C. to 600° C. for 3 hours or until the carbon is all burned off. After cooling, add 2 milliliters of hydrochloric acid, and slowly evaporate to dryness on a steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 milliliters of hot water, and digest for 2 minutes. Add, dropwise, ammonia T.S. until the solution is just alkaline to litmus paper, then add, dropwise, diluted acetic acid until the solution is slightly acid to litmus paper, and then add an excess of 2 milliliters of the diluted acetic acid. Filter if necessary, wash the crucible and the filter with about 10 milliliters of water, and dilute to exactly 25 milliliters.

(iii) *Procedure.* Place in a 50-milliliter Nessler tube 2 milliliters of diluted acetic acid and exactly the quantity of standard lead solution containing the lead equivalent of the heavy metals limit specified for the substance to be tested and add water to make 25 milliliters. Transfer the 25-milliliter solution of the sample, subdivision (ii) of this subparagraph, to a 50-milliliter Nessler tube, and add 10 milliliters of hydrogen sulfide T.S. to each tube, mix, allow to stand

for 10 minutes, and view downward over a white surface; the color of the solution of the sample should be no darker than the standard that contains the lead equivalent of the heavy metals limit of the test.

(10) *Identity.* Dissolve about 10 milligrams of oleandomycin phosphate in 5 milliliters of hydrochloric acid and heat the solution in a water bath; a greenish-yellow color is produced.

(Sec. 507, 59 Stat. 463, as amended, 76 Stat. 785, 786, 787; 21 U.S.C. 357)

Effective date. This order shall become effective 60 days from the date of its publication in the FEDERAL REGISTER.

(Sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357)

Dated: June 2, 1964.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 64-5686; Filed, June 12, 1964; 8:45 a.m.]

TESTS AND METHODS OF ASSAY AND CERTIFICATION OF ANTIBIOTIC DRUGS SUBJECT TO DRUG AMENDMENTS OF 1962

The Commissioner of Food and Drugs has evaluated the views and comments received in response to the notice of proposed rulemaking published in the FEDERAL REGISTER of May 2, 1963 (28 F.R. 4382), and has concluded that the following regulations should issue for the certification of certain antibotic drugs subject to the Drug Amendments of 1962 (76 Stat. 785–787; Public Law 87–781). Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (21 CFR 2.90; 29 F.R. 471), Title 21 is amended as hereinafter set forth. Included in this order are three sections (148e.29, 148e.30, and 148e.31) not included in any prior notice. Section 148e.29 *Erythromycin ethylsuccinate chewable tablets* and § 148e.30 *Erythromycin estolate chewable tablets* are new dosage forms developed since publication of the notice of proposed rulemaking of May 2, 1963. These new erythromycin preparations have been found to be safe and efficacious, conditions prerequisite to their certification, and are therefore incorporated in the part assigned to other erythromycin preparations. The information on which § 148e.31 *Erythromycin ophthalmic ointment* is based was included in the notice of proposed rulemaking of May 2, 1963, under § 148e.14 *Erythromycin ointment.* As proposed, § 148e.14 included erythromycin topical ointment and erythromycin ophthalmic ointment. In this order the two types of ointment are assigned individual monographs.

PART 146—GENERAL REGULATIONS FOR THE CERTIFICATION OF ANTIBIOTIC AND ANTIBIOTIC-CONTAINING DRUGS

1. Section 146.1 is amended in the following respects:

a. By adding to paragraph (c) (1) the following new subdivisions:

§ 146.1 Definitions and interpretations applicable to all certifiable antibiotic drugs.

* * * * * *

(c) * * *

(1) * * *

(iii) The term "unit" applied to nystatin means the nystatin activity (potency) contained in 0.2817 microgram of the nystatin master standard when dried for 2 hours at 40° C. and a pressure of 5 millimeters or less.

(iv) The term "unit" applied to polymyxin B means the polymyxin activity (potency) contained in 1.2739 micrograms of the polymyxin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

b. By adding to paragraph (c) (2) the following new subdivisions:

(c) * * *

(2) * * *

(x) The term "microgram" applied to amphomycin means the amphomycin activity (potency) contained in 0.9355 microgram of the amphomycin master standard when dried for 4 hours at 60° C. and a pressure of 5 millimeters or less.

(xi) The term "microgram" applied to amphotericin A means the amphotericin A activity (potency) contained in 1.0 microgram of the amphotericin A master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xii) The term "microgram" applied to amphotericin B means the amphotericin B activity (potency) contained in 1.124 micrograms of the amphotericin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xiii) The term "microgram" applied to colistin means the colistin base activity (potency) contained in 1.495 micrograms of the colistin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xiv) The terms "microgram" applied to colistimethate means the activity (potency) calculated as colistin base that is contained in 1.938 micrograms of the colistimethate master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xv) The term "microgram" applied to cycloserine means the cycloserine activity (potency) contained in 1.0 microgram of the cycloserine master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xvi) The term "microgram" applied to erythromycin means the erythromycin base activity (potency) contained in 1.02 micrograms of the erythromycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xvii) The term "microgram" applied to gramicidin means the gramicidin activity (potency) contained in 1.0 microgram of the gramicidin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xviii) The term "microgram" applied to griseofulvin means the griseofulvin activity (potency) contained in 1.0 microgram of the griseofulvin master standard.

(xix) The term "microgram" applied to kanamycin means the kanamycin base activity (potency) contained in 1.299 micrograms of the kanamycin master standard.

(xx) The term "microgram" applied to neomycin means the neomycin base activity (potency) contained in 1.429 micrograms of the neomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xxi) The term "microgram" applied to novobiocin means the novobiocin acid activity (potency) contained in 1.033 micrograms of the novobiocin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xxii) The term "microgram" applied to oleandomycin means the oleandomycin base activity (potency) contained in 1.176 micrograms of the oleandomycin master standard.

(xxiii) [Reserved]

(xxiv) The term "microgram" applied to oxytetracycline means the oxytetracycline base activity (potency) contained in 1.13 micrograms of the oxytetracyline master standard.

(xxv) The term "microgram" applied to paromomycin means the paromomycin base activity (potency) contained in 1.333 micrograms of paromomycin master standard.

(xxvi) The term "microgram" applied to tyrothricin means the activity (potency) contained in 0.2 microgram of the gramicidin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xxvii) The term "microgram" applied to vancomycin means the vancomycin base activity (potency) contained in 1.25 micrograms of the vancomycin master standard.

(xxviii) The term "microgram" applied to viomycin means the viomycin base activity (potency) contained in 1.355 micrograms of the viomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

c. By adding to paragraph (d) the following new subparagraphs:

(d) * * *

(2) The term "amphotericin A working standard" means a specific lot of a homogeneous preparation of amphotericin A.

(3) The term "amphotericin B working standard" means a specific lot of a homogeneous preparation of amphotericin B.

* * * * * *

(12) The term "amphomycin working standard" means a specific lot of a homogeneous preparation of amphyomycin.

(13) The term "colistin working standard" means a specific lot of a homogeneous preparation of colistin.

(14) The term "colistimethate working standard" means a specific lot of a homogeneous preparation of colistimethate.

(15) The term "cycloserine working standard" means a specific lot of a homogeneous preparation of cycloserine.

(16) The term "erythromycin working standard" means a specific lot of a homogeneous preparation of erythromycin.

(17) The term "gramicidin working standard" means a specific lot of a homogeneous preparation of gramicidin.

(18) The term "griseofulvin working standard" means a specific lot of a homogeneous preparation of griseofulvin.

(19) The term "kanamycin working standard" means a specific lot of a homogeneous preparation of kanamycin.

(20) The term "neomycin working standard" means a specific lot of a homogeneous preparation of neomycin.

(21) The term "novobiocin working standard" means a specific lot of a homogeneous preparation of novobiocin.

(22) The term "nystatin working standard" means a specific lot of a homogeneous preparation of nystatin.

(23) The term "oleandomycin working standard" means a specific lot of a homogeneous preparation of oleandomycin.

(24) [Reserved]

(25) The term "oxytetracycline working standard" means a specific lot of a homogeneous preparation of oxytetracycline.

(26) The term "paromomycin working standard" means a specific lot of a homogeneous preparation of paromomycin.

(27) The term "polymyxin B working standard" means a specific lot of a homogeneous preparation of polymyxin B.

(28) The term "vancomycin working standard" means a specific lot of a homogeneous preparation of vancomycin.

(29) The term "viomycin working standard" means a specific lot of a homogeneous preparation of viomycin.

d. By changing paragraph (g) and the introduction to paragraph (h) to read:

(g) Except as specifically provided by the regulations in this chapter, no provision of any regulation shall be construed as exempting any certifiable antibiotic drug from any applicable provision of the act or any regulation thereunder.

(h) The regulations in this chapter, prescribing tests and methods of assay for antibiotic and antibiotic-containing drugs, shall not be construed as preventing the Commissioner from using any other test or method of assay in his investigations to determine whether or not:

2. Title 21, Chapter I, is amended by adding thereto the following new parts: [1]

PART 148e—ERYTHROMYCIN

Sec.

148e.1 Erythromycin.
148e.2 Erythromycin ethylcarbonate.
148e.3 Erythromycin gluceptate.
148e.4 [Reserved]
148e.5 Erythromycin estolate.
148e.6 Erythromycin stearate.
148e.7 Erythromycin ethylsuccinate.
148e.8 Erythromycin sulfate.
148e.9 Erythromycin ethylsuccinate-sulfanilamide dental powder.
148e.10 Erythromycin ethylsuccinate granules for oral suspension.
148e.11 Erythromycin ethylcarbonate for oral suspension.
148e.12 Erythromycin estolate for oral suspension.
148e.13 Erythromycin ethylsuccinate oral suspension.
148e.14 Erythromycin ointment.
148e.15 Erythromycin-neomycin sulfate ointment.
148e.16 Erythromycin-polymyxin β sulfate ointment.
148e.17 Erythromycin estolate capsules.
148e.18 [Reserved]
148e.19 Erythromycin gluceptate dental cones.
148e.20 Erythromycin gluceptate-polymyxin β sulfate-benzocaine for otic solution.
148e.21 Erythromycin sulfate-polymyxin β sulfate-pramoxine hydrochloride otic solution.
148e.22 Erythromycin estolate for pediatric drops.
148e.23 [Reserved]
148e.24 Erythromycin enteric-coated tablets.
148e.25 Erythromycin lactobionate for intravenous infusion.
148e.26 Erythromycin tablets.
148e.27 Erythromycin stearate tablets.
148e.28 Erythromycin estolate tablets with sulfonamides.
148e.29 Erythromycin ethylsuccinate chewable tablets.
148e.30 Erythromycin estolate chewable tablets.
148e.31 Erythromycin ophthalmic ointment.

AUTHORITY: The provisions of this Part 148e issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C. 357.

§ 148e.1 Erythromycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin is the odorless, white to grayish-white or slightly yellow crystalline compound of a kind of erythromycin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It contains not less than 850 micrograms of erythromycin per milligram calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 10 percent.

(iv) Its pH in a saturated aqueous solution, prepared by using 100 milligrams of erythromycin per milliliter, is not less than 8.0 nor more than 10.5.

(v) Its residue on ignition is not more than 2.0 percent.

(vi) Its heavy metals content is not more than 50 parts per million.

(vii) It gives a positive identity test for erythromycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, residue on ignition, heavy metals, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 500 milligrams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency* (i) *Cylinders (cups).* Use cylinders described under § 141a.1(a) of this chapter.

(ii) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make nutrient agar as follows:

(a) For carrying the test organism:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(b) For base and seed layers: Same formulation as in (a), except adjust the medium to pH 8.0 after sterilization.

In lieu of preparing the media from the individual ingredients specified in this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the individual ingredients specified in this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Working standard.* Dry approximately 30 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient methyl alcohol to give a concentration of 10,000 micrograms of erythromycin base per milliliter. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of 1,000 micrograms of erythromycin base per milliliter. This stock solution may be kept under refrigeration for 14 days.

(iv) *Preparation of sample.* Dissolve an accurately weighed amount of the sample (approximately 50 milligrams) in approximately 5.0 milliliters of methyl alcohol and further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to make a solution containing 1.0 milligram of erythromycin base per milliliter (estimated). Further dilute an aliquot to the reference point of the assay with 0.1*M* potassium phosphate buffer, pH 8.0.

(v) *Preparation of test organism.* The test organism is *Sarcina lutea* (ATCC 9341), which is maintained on slants of agar described under (ii) (a) of this subparagraph. Wash the organism from the agar slant with 3 milliliters of sterilized U.S.P. saline T.S. onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in (ii) (a) of this subparagraph. Spread the suspension of organism over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C.–35° C., and then wash the resulting growth from the agar surface with about 50 milliliters of sterilized U.S.P. saline T.S. Standardize the suspension so that a 1:40 dilution of it will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the bulk suspension (usually 1.5 milliliters) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in (ii) (b) of this subparagraph to each Petri dish (20 millimeters x 100

[1] Certain portions of the proposal will be covered by a subsequent order.

millimeters). Distribute the agar evenly on the plates and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in (ii) (b) of this subparagraph, cool to 48° C., add the proper amount of the test organism as described in (v) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly on the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place six cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare daily in 0.1*M* potassium phosphate buffer, pH 8.0, from the stock solution described in (iii) of this subparagraph, concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms of erythromycin per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution except the 1.0 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the point tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram per milliliter concentration is 16.5 millimeters and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is 0.2 millimeter. If the average readings of the 0.8 microgram per millimeter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5}$$

$$H=\frac{3e+2d+c-a}{5}$$

where:

L=Calculated zone diameter for the lowest concentration of the standard curve;

H=Calculated zone diameter for the highest concentration of the standard curve;

c=Average zone diameter of 36 readings of the 1.0 micrograms per milliliter standard;

a, b, d, e=Corrected average values for 0.64, 0.80, 1.25, and 1.56 micrograms per milliliter solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 1.0 microgram per milliliter solution and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the standard curve, read the potencies corresponding to the corrected values of zone sizes.

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer orally 1.0 milliliter of a suspension of the drug containing 30 milligrams of erythromycin per milliliter in 10-percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Using a saturated aqueous solution (100 milligrams of erythromycin per milliliter) proceed as directed in § 141a.5(b) of this chapter.

(5) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(6) *Heavy metals.* Proceed as described in § 148m.1(b) (9) of this chapter.

(7) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(8) *Identity test.* The infrared absorption, in the spectrum from 2 microns to 12 microns, of a 5 percent solution of the sample in chloroform compares qualitatively to that of the erythromycin working standard similarly treated.

§ 148e.2 Erythromycin ethylcarbonate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylcarbonate is a white, crystalline ester of erythromycin obtained by the reaction of a kind of erythromycin and chloroethylcarbonate. It is slightly soluble in water, freely soluble in alcohol, methyl alcohol, acetone, ether, and chloroform. It is so purified and dried that:

(i) It contains not less than 775 micrograms of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 8 percent.

(iv) Its pH is not less than 6.3 and not more than 8.0.

(v) It gives a positive identity test for erythromycin ethylcarbonate.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 containers, each containing not less than 300 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148e.1 (b) (1), except to prepare the sample, wash an accurately weighed amount of the sample (approximately 50 milligrams) into a 100-milliliter volumetric flask, using approximately 40 milliliters of absolute methyl alcohol. Immediately dilute to volume with sterile distilled water. Place this solution in a 60° C. water bath for 3 hours or allow it to remain at room temperature for 40 hours to 48 hours to accomplish hydrolysis. Adjust the volume to 100 milliliters with sterilized distilled water and mix well. Further dilute an aliquot to the reference point of the assay with 0.1*M* potassium phosphate buffer, pH 8.0.

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer 0.5 milliliter of a suspension containing 100 milligrams of erythromycin per milliliter in a 10 percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a concentration of 200 milligrams per milliliter.

(5) *Identity tests.* Proceed as directed in § 148e.1(b) (8), except that an authentic erythromycin ethylcarbonate is used as the sample of comparison.

(6) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148e.3 Erythromycin gluceptate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin gluceptate is the white powder of the glucoheptonic acid salt of erythromycin or a mixture of two or more such salts. It is freely soluble in water, alcohol, and methyl alcohol. It is slightly soluble in acetone and chloroform, but is practically insoluble in ether. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5.0 percent.

(vi) Its pH in an aqueous solution containing 25 milligrams per milliliter is not less than 6.0 nor more than 8.0.

(vii) It gives a positive identity test for erythromycin gluceptate.

(2) *Packaging.* In addition to the requirements of § 148.2 of this chapter if it is packaged for dispensing and intended for intravenous use, it shall contain the equivalent of 250 milligrams, 500 milligrams, or 1.0 gram of erythromycin per vial.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(4) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing not less than 300 milligrams.

(2) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers of the batch.

(2) For sterility: 10 immediate containers of the batch.

(5) *Fees.* $5.00 for each container submitted in accordance with subparagraph (4) (ii) (*a*) (*1*) and (*b*) (*1*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (4) (ii) (*a*) (2) and (*b*) (2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1(b) (1), except when packaged for dispensing dissolve the sample as directed in the labeling and further dilute with 0.1*M* potassium phosphate buffer, pH 8.0. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent nor more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Sterility.* Use 500 milligrams from each container tested, and proceed as directed in § 141a.2 of this chapter, except that neither the penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1 milliliter per kilogram of a solution in pyrogen-free distilled water containing 30 milligrams of erythromycin per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution in sterilized distilled water containing 2 milligrams of erythromycin base per milliliter.

(5) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a concentration of 25 milligrams per milliliter.

(7) *Identity.* The infrared absorption spectrum of a liquid petrolatum dispersion compares qualitatively to that of a similarly treated authentic sample of erythromycin gluceptate.

§ 148e.4 [Reserved]

§ 148e.5 Erythromycin estolate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate is the lauryl sulfate salt of the propionyl ester of a kind of erythromycin or a mixture of two or more such salts. It occurs as a white crystalline powder. It is soluble in alcohol, methyl alcohol, acetone, and chloroform, but is practically insoluble in water. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 4.0 percent.

(iv) Its pH is not less than 4.5 nor more than 7.0.

(v) It gives positive identity tests for erythromycin estolate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, identity, and crystallinity.

(ii) Samples of the batch: A minimum of 10 containers, each containing not less than 300 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (*1*) of this chapter, except to prepare the sample transfer approximately 100 milligrams, accurately weighed, to a 100-milliliter volumetric flask. Dissolve in 40 milliliters of absolute methyl alcohol and immediately dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0. Hydrolyze this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours.

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer 0.5 milliliter of a suspension containing 40 milligrams of erythromycin per milliliter in a 10 percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous suspension containing 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(6) *Identity test.* Proceed as directed in § 148e.1(b) (8), except compare the infrared absorption spectrum with that of an authentic sample of erythromycin estolate.

§ 148e.6 Erythromycin stearate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate is the odorless white or slightly yellow crystalline powder of the stearic acid salt of erythromycin. It is practically insoluble in water but is soluble in alcohol, methyl alcohol, chloroform, and ether. It is so purified and dried that:

(i) It contains not less than 500 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5.0 percent.

(iv) Its pH is not less than 6.0 and not more than 11.0.

(v) Its residue on ignition is not more than 2.0 percent.

(vi) It gives positive identity tests for erythromycin stearate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples required: A minimum of 10 containers, each consisting of 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1), except prepare the sample in the following manner: Dissolve 50 milligrams, accurately weighed, in sufficient absolute methyl alcohol to give an estimated concentration of 1 milligram of erythromycin per milliliter.

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer 0.5 milliliter of a suspension containing 80 milligrams of erythromycin per milliliter, prepared by grinding the sample in sterile distilled water, using 1 drop of polysorbate 80 per 3 grams of sample.

(3) *Moisture content.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 1 percent slurry of erythromycin stearate in water.

(5) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(6) *Identity.* Proceed as directed in § 148e.1(b) (8), except compare qualitatively the infrared absorption spectrum with that of an authentic sample of erythromycin stearate.

(7) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148e.7 Erythromycin ethylsuccinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate is the white, odorless, crystalline ethylsuccinate ester of erythromycin. It is so purified and dried that:

(i) It contains not less than 765 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) Its moisture content is not more than 3.0 percent.

(v) Its pH is not less than 6.0 and not more than 8.5.

(vi) Its residue on ignition is not more than 1.0 percent.

(vii) It gives a positive identity test for erythromycin ethylsuccinate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 immediate containers.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (*a*) of this paragraph; $10.00 for all immediate containers submitted in accordance with subparagraph (3) (ii) (*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1), except prepare the sample in the following manner: Dissolve approximately 50 milligrams, accurately weighed, in sufficient absolute methyl alcohol to give an estimated concentration of 1 milligram of erythromycin per milliliter. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0.

(2) *Sterility.* Using approximately 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer 0.5 milliliter of a suspension in sterile distilled water containing 80 milligrams of erythromycin per milliliter.

(4) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(5) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 1.0 percent suspension in water.

(6) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(7) *Identity.* Using a suitable spectrophotometer with 0.1-millimeter cells, scan the infrared absorption spectrum between 2 microns and 12.5 microns of a 5 percent chloroform solution of erythromycin ethylsuccinate. The absorption spectrum compares qualitatively to that of an authentic sample of erythromycin ethylsuccinate.

(8) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148e.8 Erythromycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin sulfate is the sulfate salt of erythromycin. It is an odorless, practically white, crystalline powder. It is so purified and dried that:

(i) It contains not less than 840 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) Its moisture content is not more than 7.0 percent.

(iii) Its pH in a solution containing 10 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It gives a positive identity test for erythromycin sulfate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, and identity.

(ii) Samples required: 10 containers, each consisting of 300 milligrams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1(b)(1), except prepare the sample in the following manner: Dissolve approximately 50 milligrams, accurately weighed, in sufficient absolute methyl alcohol to give a concentration of 1.0 milligram of erythromycin per milliliter.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a concentration of 10 milligrams per milliliter.

(4) *Identity.* Proceed as directed in § 148e.1(b)(8), except that its infrared absorbtion spectrum compares qualitatively to that of a similarly prepared authentic sample of erythromycin sulfate.

§ 148e.9 Erythromycin ethylsuccinate-sulfanilamide dental powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate-sulfanilamide dental powder is a dry mixture of crystalline erythromycin ethylsuccinate, sulfanilamide, and suitable and harmless flavorings. Each vial contains the equivalent of 10 milligrams of erythromycin and 250 milligrams of sulfanilamide. It is sterile. Its moisture content is not more than 1.0 percent. The crystalline erythromycin ethylsuccinate used conforms to the standards of § 148e.7(a)(1) (i), (iii), (iv), (v), (vi), and (vii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, toxicity, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 containers, each consisting of 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 30 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One immediate container of each containing 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph; $0.75 for each container submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3) (ii) (*b*) (2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1), using a representative number of immediate containers to prepare the sample for assay. Its potency is satisfactory if it contains not less than 90 percent and not more than 135 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Sterility.* Use the entire contents of each container tested, and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148e.10 Erythromycin ethylsuccinate granules for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate granules for oral suspension is a dry mixture of erythromycin ethylsuccinate, with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It contains the equivalent of 40 milligrams of erythromycin per milliliter of the reconstituted suspension. Its moisture content is not more than 1 percent. When reconstituted as directed in the labeling, its pH is not less than 7.0 nor more than 9.0. The crystalline erythromycin ethylsuccinate used conforms to the standards prescribed by § 148e.7(a) (i), (iii), (iv), (v), (vi), and (vii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. The expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, toxicity, moisture, pH, residue on ignition, identity, and crystallinity.

(b) The batch, for potency, pH, and moisture.

(ii) Samples required:

(a) The erythromycin ethylsuccinate used in making the batch: 10 containers each consisting of approximately 500 milligrams.

(b) The batch: A minimum of 6 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1), except prepare the sample in the following manner: Reconstitute the sample as directed in the labeling. Transfer 2 milliliters of the reconstituted suspension to a 200-milliliter volumetric flask, add absolute methyl alcohol to volume, and mix well. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0. Its potency is satisfactory if it contains not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the suspension prepared as directed in the labeling.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148e.11 Erythromycin ethylcarbonate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylcarbonate for oral suspension is a dry mixture of erythromycin ethylcarbonate, with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It may contain suitable sulfonamides. The erythromycin ethylcarbonate content is not less than 40 milligrams of erythromycin per milliliter of the reconstituted suspension. Its moisture content is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 6.4 and not more than 7.0. The erythromycin ethylcarbonate used conforms to the standards prescribed by § 148e.2 (a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) Erythromycin ethylcarbonate used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(b) The batch for potency, moisture, and pH.

(ii) Samples required:

(a) Erythromycin ethylcarbonate used in making the batch: 10 containers, each containing 500 milligrams.

(b) The batch: A minimum of 6 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One container of each, consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.2 (b) (1), except prepare the sample in the following manner: Reconstitute the suspension as directed in the labeling. Place an aliquot (usually a single dose) of an accurately measured volume of the oral suspension in a 100-milliliter volumetric flask and dissolve in 40 milliliters of reagent-grade methyl alcohol. Bring to volume with distilled water and mix well. Hydrolyze either in a 60° C. water bath for 3 hours or keep at room temperature for 40 hours to 48 hours. Make subsequent dilutions in 0.1*M* potassium phosphate buffer, pH 8.0. The erythromycin ethylcarbonate content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5 (b) of this chapter, using the suspension prepared as directed in its labeling.

§ 148e.12 Erythromycin estolate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate for oral suspension is a dry mixture of erythromycin estolate, with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It may contain suitable sulfonamides. The erythromycin estolate content is 25 milligrams of erythromycin per milliliter of the reconstituted suspension. When reconstituted as directed in its labeling, its pH is not less than 5.0 and not more than 7.0. Its moisture content is not more than 2.0 percent. The erythromycin estolate used conforms to the standards of § 148e.5(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin estolate used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(b) The batch: Potency, moisture, and pH.

(ii) Samples required:

(a) The erythromycin estolate used in making the batch: 10 immediate containers, each consisting of 300 milligrams.

(b) The batch: A minimum of 6 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One container of each, consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.5 (b) (1), except prepare the sample in the following manner: Reconstitute the suspension as directed in the labeling. Place an accurately measured aliquot (usually a single dose) of the well-shaken suspension in a 100-milliliter volumetric flask and dissolve with 40 milliliters of reagent-grade methyl alcohol. Bring to volume with 0.1*M* potassium phosphate buffer, pH 8.0, and mix well. Hydrolyze at 60° C. for 2 hours or at room temperature for 16 hours to 18 hours.

The erythromycin estolate content is satisfactory if it is not less than 90 percent nor more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, using the dry powder.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the drug reconstituted as directed in its labeling.

§ 148e.13 Erythromycin ethylsuccinate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate oral suspension is erythromycin ethylsuccinate, with suitable and harmless buffer substances, dispersing agents, colorings, and flavorings suspended in peanut oil and glycerin. It contains the equivalent of not less than 40 milligrams of erythromycin per milliliter. Its moisture content is not more than 5.0 percent. The crystalline erythromycin ethylsuccinate used conforms to the standards prescribed by § 148e.7(a) (1) (i), (iii), (iv), (v), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirement of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin ethylsuccinate used in making the batch for potency, toxicity, moisture, pH, identity, residues on ignition, and crystallinity.

(b) The batch for potency and moisture:

(ii) Samples required:

(a) The erythromycin ethylsuccinate used in making the batch: 10 containers, each consisting of 500 milligrams.

(b) The batch: A minimum of 5 immediate containers.

(c) In case of an initial request for certification, each other ingredient used

in making the batch: One container of each, consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1), except prepare the sample in the following manner: by means of a syringe, transfer 2 milliliters of the sample to a 100-milliliter volumetric flask. Add absolute methyl alcohol to volume, and mix well. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.14 Erythromycin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ointment is erythromycin in a suitable and harmless ointment base. It may contain suitable preservatives. Each gram of ointment contains 10 milligrams of erythromycin. The moisture content is not more than 1.0 percent. The erythromycin used conforms to the standards prescribed by § 148e.1(a) (1) (i), (iii), (iv), (v), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each immediate container or package submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1), except prepare the sample by any one of the following procedures:

(i) Place 1 gram of the ointment, accurately weighed, in a 1-liter volumetric flask, add 50 milliliters of peroxide-free ether, and shake until dissolved. Add 200 milliliters of absolute methyl alcohol, and mix thoroughly. Fill to volume and further dilute to the reference point with 0.1*M* potassium phosphate buffer, pH 8.0.

(ii) Place 1 gram of the ointment, accurately weighed, in a 250-milliliter separatory funnel containing 50 milliliters of reagent-grade petroleum ether. Shake until dissolved. Wash with four separate washings of a 4:1 mixture of reagent-grade methyl alcohol and distilled water. Combine the washings and bring to volume with the methyl alcohol-water solution in a volumetric flask. Further dilute to the reference point in 0.1*M* potassium phosphate buffer, pH 8.0.

(iii) Place 1 gram of the ointment, accurately weighed, in a high-speed blender with 100 milliliters of polyethylene glycol 400, and blend for 3 minutes. Filter through a cotton plug or filter paper and further dilute in 0.1*M* potassium phosphate buffer, pH 8.0.

Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.15 Erythromycin-neomycin sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin-neomycin sulfate ointment is erythromycin and neomycin sulfate with a suitable emollient and preservatives, in a suitable and harmless ointment base. Each gram contains 5 milligrams of erythromycin and 3.5 milligrams of neomycin. The moisture content is not more than 1 percent. The erythromycin used conforms to the standards prescribed therefor by § 148e.1(a) (1) (i), (iii), (iv), (v), and (vii). The neomycin sulfate used conforms to the standards prescribed in § 148i.1(a) (1) (i), (vi) (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, pH, moisture, and identity.

(*c*) The batch for erythromycin content, neomycin content, and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each container submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin content.* Proceed as directed in § 148e.14(b) (1) (iii). Its erythromycin content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain.

(ii) *Neomycin content.* Proceed as directed in § 148i.3(b) (1) of this chapter, except that after the extraction has been completed and the volume adjusted, place the buffer solution in a second separatory funnel and wash with three 30-milliliter portions of peroxide-free ether. Discard the ether washes. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.16 Erythromycin-polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin-polymyxin B sulfate ophthalmic ointment is erythromycin and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains 5 milligrams of erythromycin and 10,000 units of polymyxin B. The moisture content is not more than 1 percent. The crystalline erythromycin used conforms to the standards prescribed therefor by § 148e.1(a) (1) (i), (iii), (iv), (v), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a) (1) (i), (v), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, moisture, and identity.

(*c*) The batch for erythromycin content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(c) The batch: Six immediate containers.

(d) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3) (ii) (a), (b), and (d) of this paragraph; $5.00 for each container submitted in accordance with subparagraph (3) (ii) (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin content.* Proceed as directed in § 148e.14(b) (1). The erythromycin content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in § 148p.3(b) (1) of this chapter. The polymyxin B content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.17 Erythromycin estolate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate capsules are capsules containing erythromycin estolate, with suitable and harmless buffer substances and diluents, enclosed in a gelatin capsule. The erythromycin estolate content of each capsule is equivalent to either 250 milligrams of erythromycin or 125 milligrams of erythromycin. The moisture content is not more than 5 percent. The erythromycin estolate used conforms to the standards prescribed therefor by § 148e.5(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin estolate used in making the batch for potency, toxicity, pH, moisture, crystallinity, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(c) In case of an initial request for certification, each other ingredient used: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (a) and (c) of this paragraph; $0.75 for each capsule submitted in accordance with subparagraph (3) (ii) (b) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.5 (b) (1), except prepare the sample in the following manner: Using 200 milliliters of reagent-grade methyl alcohol and 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, blend a representative number of capsules in a high-speed glass blender for 2 to 3 minutes. The erythromycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

§ 148e.19 Erythromycin gluceptate dental cones.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin gluceptate dental cones are composed of erythromycin gluceptate and suitable and harmless binders and diluents. The erythromycin gluceptate content of each cone is equivalent to 5 milligrams of erythromycin. The moisture content is not more than 1.5 percent. The crystalline erythromycin gluceptate used conforms to the standards prescribed by § 148e.3(a) (1) (i), (iii), (v), (vi), and (vii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin gluceptate used, for potency, toxicity, moisture, pH, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The erythromycin gluceptate used: 10 packages, each containing 300 milligrams.

(b) The batch: A minimum of 30 cones.

(c) In case of an initial request for certification, each other ingredient used: One package of each, containing 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (a) and (c) of this paragraph; $0.75 for each cone submitted in accordance with subparagraph (3) (ii) (b) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.3(b) (1), except prepare the sample as follows: Blend a representative number of cones in a high-speed glass blender with 500 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0. The erythromycin gluceptate content is satisfactory if it is not less than 90 percent nor more than 135 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

§ 148e.20 Erythromycin gluceptate-polymyxin B sulfate-benzocaine for otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin gluceptate-polymyxin B sulfate-benzocaine for otic solution is a packaged combination of one immediate container of dry erythromycin gluceptate powder and one immediate container of polymyxin B sulfate and benzocaine, dissolved in propylene glycol. When reconstituted as directed in its labeling, each milliliter contains the equivalent of 5 milligrams of erythromycin, 10,000 units of polymyxin B, and 50 milligrams of benzocaine. The moisture content of the dry powder is not more than 5.0 percent. The moisture content of the diluting fluid is not more than 1.5 percent. The erythromycin gluceptate used conforms to the standards prescribed by § 148e.3 (a) (1) (i), (v), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a) (1) (i), (v), (vi), (vii), and (ix) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefore by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin gluceptate used in making the batch, for potency, moisture, pH, and identity.

(b) The polymyxin B sulfate used in making the batch, for potency, moisture, pH, residue on ignition, and identity.

(c) The batch for erythromycin content, polymyxin B content, moisture content of the erythromycin gluceptate dry powder, and moisture content of the diluting fluid.

(ii) Samples required:

(a) The erythromycin gluceptate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(c) The batch:

(1) For all tests except moisture: A minimum of 6 combined packages.

(2) For moisture content: A minimum of 3 combined packages.

(d) In case of an initial request for certification, each other ingredient used: One package consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin content.* Proceed as directed in § 148e.3(b) (1),

using the entire contents of each vial of erythromycin gluceptate. The erythromycin content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain.

(ii) *Polymyxin B content.* By means of a needle and syringe, withdraw a representative aliquot of the diluting fluid, and proceed as directed in § 148p.1(b)(1) of this chapter. The polymyxin B content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Moisture*—(i) *Erythromycin gluceptate vial.* Proceed as directed in § 141a.26(e) of this chapter, using the erythromycin gluceptate dry powder.

(ii) *Polymyxin B diluting fluid.* Proceed as directed in § 141a.8(b) of this chapter, using the diluting fluid without reconstitution.

§ 148e.21 Erythromycin sulfate-polymyxin B sulfate-pramoxine hydrochloride otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin sulfate-polymyxin B sulfate-pramoxine hydrochloride otic solution is erythromycin sulfate, polymyxin B sulfate, and pramoxine hydrochloride dissolved in a suitable and harmless vehicle. Each milliliter contains the equivalent of 5 milligrams of erythromycin, 10,000 units of polymyxin B sulfate, and 10 milligrams of pramoxine hydrochloride. It contains not more than 1.5 percent moisture. The erythromycin sulfate used conforms to the standards prescribed by § 148e.8(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1) (i), (v), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin sulfate used in making the batch for potency, moisture, pH, identity, and crystallinity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The batch for erythromycin content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The erythromycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: 1 container of each, containing not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each container submitted in accordance with subparagraph (3)(ii)(*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin content.* Proceed as directed in § 148e.1(b)(1), using a 1.0 milliliter aliquot of the solution. The erythromycin sulfate content is satisfactory if it contains not less than 90 percent and not more than 135 percent of the number of milligrams of erythromycin that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in § 148p.1(b)(1) of this chapter, using a 1.0-milliliter aliquot of the solution. The polymyxin B content is satisfactory if it contains not less than 90 percent and not more than 135 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.22 Erythromycin estolate for pediatric drops.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate for pediatric drops is a dry mixture of erythromycin estolate, with suitable and harmless dispersing agents, buffer substances, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains the equivalent of 100 milligrams of erythromycin. Its moisture content is not more than 2.0 percent. Its pH is not less than 5.0 nor more than 5.5. The erythromycin estolate used conforms to the standards prescribed by § 148e.5(a)(1). Each other ingredient used, if its name is recognized in the U.S.P. or the N.F., conforms with the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, toxicity, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each package or immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.12(b)(1). Its potency is satisfactory if it contains not less than 90 percent nor more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the suspension prepared as directed in the labeling.

§ 148e.23 [Reserved]

§ 148e.24 Erythromycin enteric-coated tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength quality, and purity.* Erythromycin enteric-coated tablets are enteric-coated tablets composed of erythromycin, suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. It may contain suitable sulfonamides. The potency of each tablet is 100 milligrams of erythromycin or 250 milligrams of erythromycin. Each tablet shall meet the tests for enteric-coated tablets set forth in the U.S.P. and shall disintegrate within a total time of 2 hours. The moisture content is not more than 6 percent. The erythromycin base used in making the batch conforms to the standards of § 148e.1(a)(1) (i), (ii), (iii), (iv), (v), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, toxicity, moisture, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration time: 6 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.26(b)(1). The erythromycin content is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *Disintegration time.* Proceed as directed in the U.S.P. for enteric-coated tablets.

§ 148e.25 Erythromycin lactobionate for intravenous infusion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin lactobionate for intravenous infusion is a dry mixture of erythromycin lactobionate and a suitable preservative. It contains the equivalent of either 300 milligrams of erythromycin or 1 gram of erythromycin per vial. It is sterile. It is nonpyrogenic. It is nontoxic. Its moisture content is not more than 5 percent. Its pH is not less than 6.5 and not more than 7.5. The erythromycin used conforms to the standards prescribed by § 148e.1(a)(1) (i), (iii), (iv), (v), and (vi). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of §148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, heavy metals, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, toxicity, moisture, pH, and identity.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 containers, each consisting of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One container of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $5.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Dissolve the entire contents of the vial in sufficient distilled water to give a concentration of 10 milligrams of erythromycin per milliliter (estimated). Proceed as directed in § 148e.1(b)(1). The erythromycin lactobionate content is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Sterility.* Use the entire contents or not more than 500 milligrams from each container tested, and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1 milliliter per kilogram of a solution in sterile, pyrogen-free distilled water containing 30 milligrams of erythromycin per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of a solution in sterile distilled water containing 3 milligrams of erythromycin per milliliter.

(5) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a concentration of 50 milligrams of erythromycin per milliliter.

(7) *Identity.* The infrared absorption spectrum between 2 microns and 12 microns of a liquid petrolatum dispersion compares qualitatively to that of a similarly prepared authentic erythromycin lactobionate sample.

§ 148e.26 Erythromycin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin tablets are erythromycin, with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings, and suitable preservatives. It may contain suitable sulfonamides. The potency of each tablet is 75 milligrams, or 100 milligrams, or 250 milligrams of erythromycin. Tablets shall disintegrate within 1 hour. The moisture content is not more than 7.5 percent. The erythromycin used in making the batch conforms to the standards prescribed by § 148e.1(a)(1) (i), (ii), (iii), (iv), (v), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, toxicity, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, disintegration time, and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration time: 6 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: 1 package, of each, containing not less than 5 grams.

(4) *Fees.* $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph; $4.00 for each package submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1(b)(1), except prepare the sample in the following manner: Blend a representative number of tablets in a high-speed glass blender with 100 milliliters of absolute methyl alcohol. Add 400 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, and blend again for 2 to 3 minutes. The erythromycin base content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c)(2) of this chapter.

§ 148e.27 Erythromycin stearate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate tablets are tablets composed of erythromycin stearate, with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. It may contain suitable sulfonamides. The potency of each tablet is 75 milligrams, or 100 milligrams, or 125 milligrams, or 250 milligrams of erythromycin. Tablets shall disintegrate within 1½ hours, except tablets containing sulfonamides shall disintegrate within 2½ hours. The moisture content is not more than 7 percent. The erythromycin stearate used in making the tablets conforms to the standards prescribed by § 148e.6(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin stearate used in making the batch for potency, toxicity, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin stearate used in making the batch: 10 containers, each consisting of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration time: 6 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this

paragraph; $4.00 for each package submitted in accordance with subparagraph (3)(ii)(a) and (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.26(b)(1). The erythromycin stearate content is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c)(2) of this chapter.

§ 148e.28 Erythromycin estolate tablets with sulfonamides.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate tablets with sulfonamides are tablets composed of erythromycin estolate, with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings, and suitable sulfonamides. The potency of each tablet is 125 milligrams of erythromycin. Tablets shall disintegrate within 1 hour. The moisture content is not more than 4.0 percent. The erythromycin estolate used conforms to the standards prescribed by § 148e.5(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency, disintegration time, and moisture.

(ii) Samples required:

(*a*) Erythromycin estolate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration time: 6 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package, of each containing not less than 5 grams.

(4) *Fees.* $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(2) of this paragraph; $4.00 for each package submitted in accordance with subparagraphs (3)(ii)(*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.5(b)(1), except prepare the sample in the following manner: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes in 100 milliliters of methyl alcohol. Add 400 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, and blend again for 2 to 3 minutes. The erythromycin estolate content is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148e.29 Erythromycin ethylsuccinate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate chewable tablets are tablets composed of erythromycin ethylsuccinate, suitable and harmless diluents, binders, buffers, colorings, and flavorings. The potency of each tablet is equivalent to either 100 milligrams of erythromycin or 200 milligrams of erythromycin. The moisture content is not more than 5 percent. The crystalline erythromycin ethylsuccinate used conforms to the standards prescribed by § 148e.7(a)(1)(i), (iii), (iv), (v), (vi), and (vii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with § 148.3 of this chapter. The expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, toxicity, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph; $0.75 for each tablet in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1(b)(1) of this chapter, except prepare the sample in the following manner: Blend a representative number of tablets in a high-speed glass blender with 50 milliliters of absolute methyl alcohol per tablet. Further dilute to the reference point with 0.1*M* potassium phosphate buffer, pH 8.0. Its potency is satisfactory if the tablets contain not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148e.30 Erythromycin estolate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate chewable tablets are tablets composed of erythromycin estolate, and suitable and harmless diluents, binders, buffers, colorings, and flavorings. The potency of each tablet is equivalent to 125 milligrams of erythromycin. The moisture content is not more than 4 percent. The erythromycin estolate used in making the batch conforms to the standards prescribed by § 148e.5(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with § 148.3 of this chapter. The expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph; $0.75 for each tablet in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.28(b)(1). The erythromycin estolate content is satisfactory if it is not less than 90 percent nor more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

§ 148e.31 Erythromycin ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ophthalmic ointment is erythromycin in a suitable and harmless ointment base. Each gram of ointment contains 5 milligrams of erythromycin. The moisture content is not more than 1 percent. The erythromycin used conforms to the standards prescribed by § 148e.1(a)(1)(i), (ii), (iii), (iv), (v), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of

§ 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, toxicity, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch; 10 packages, each containing 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing 5 grams.

(4) *Fees.* $4.00 for each immediate container or package of the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.14 (b) (1). Its potency is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8 (b) of this chapter.

PART 148j—NOVOBIOCIN

Sec.

148j.1 Sodium novobiocin.
148j.2 Calcium novobiocin.
148j.3 Sodium novobiocin tablets.
148j.4 Calcium novobiocin-sulfamethizole tablets.
148j.5 Calcium novobiocin oral suspension.
148j.6 [Reserved]

AUTHORITY: The provision of this Part 148j issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148j.1 Sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the crystalline monosodium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 800 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) Its moisture content is not more than 6.0 percent.

(vi) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not less than 10.0 percent and not more than 11.5 percent.

(viii) Its specific rotation in an acid-methyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(ix) When dissolved in 0.1*M* sodium borate, it demonstrates a positive color identity test in a saturated aqueous solution of *N*,2,6-trichloroquinoneimine.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, moisture, pH, residue on ignition, specific rotation, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 600 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $5.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3) (ii) (*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1 (a) of this chapter.

(ii) *Culture media.* Use ingredients that conform to the standards prescribed in the U.S.P. or N.F., if such name appears in either compendium.

(*a*) Make nutrient agar for the seed layer and for carrying the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000 ml.

pH 6.5 to 6.6 after sterilization.

(*b*) Make nutrient agar for the base layer as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000 ml.

pH 6.5 to 6.6 after sterilization.

In lieu of preparing the media from the individual ingredients specified in this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in (*a*) and (*b*) of this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Working standard.* Dissolve volumetrically a suitable weighed quantity (30 milligrams to 50 milligrams) of the working standard, dried as described in § 141a.5 (a) of this chapter, in 2 milliliters of absolute ethyl alcohol and then add sufficient 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, to give a concentration of 1,000 micrograms of novobiocin per milliliter. This stock solution may be kept for 30 days when stored under refrigeration.

(iv) *Preparation of sample.* Dissolve the accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, to give a stock solution of convenient concentration. Further dilute in 1.0 percent potassium phosphate buffer, pH 6.0, to give a final concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is *Staphylococcus epidermidis* (ATCC 12228), which is maintained on slants of agar described in subdivision (ii) (*a*) of this subparagraph. Wash the organism from a recently incubated slant with 3 milliliters of sterile U.S.P. saline T.S. onto a large agar surface, such as that provided by a Roux bottle containing 300 milliliters of agar. Use sterile glass beads to aid in spreading the organism over the entire agar surface of the Roux bottle. Incubate 16 hours to 24 hours at 32° C. to 35° C. Wash the resulting growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S. Standardize the resulting bulk suspension so that a 1:25 dilution in U.S.P. saline T.S. will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the adjusted bulk suspension (usually 1.0 milliliter) to be added to each 100 milliliters of agar that will give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) (*b*) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain tops glazed on the outside, and allow to harden. Use plates the same day they are prepared. Melt a sufficient amount of agar described in subdivision (ii) (*a*) of this subparagraph, cool to 48° C., add the proper amount of the test organism bulk suspension as described in subdivision (v) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the seed agar evenly in the plates, cover again, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 1 percent potassium phosphate buffer, pH 6.0, to obtain concentrations of 0.32, 0.40, 0.50, 0.625, and 0.78 microgram of novobiocin per milliliter. Use three plates for the determination of each point on the curve, except the 0.5 microgram per milliliter concentration, a total of 12 plates. On each of three plates, fill alternately three cylinders with the 0.5 microgram per milliliter standard, and fill the other three cylinders with the concentration under test. Thus, there will be thirty-six 0.5 microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 0.5 microgram per milliliter concentration and the readings of the point tested for each of three plates, and average also all 36 readings of the 0.5 microgram per milliliter concentra-

tions. The average of the 36 readings of the 0.5 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 0.5 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.4-microgram concentration, the average of the 36 readings of the 0.5-microgram concentration is 20.0 millimeters, and the average of the 0.5-microgram concentration of this set of three plates is 19.8 milliliters, the correction is +0.2 millimeter. If the average reading of the 0.4-microgram concentration of these same three plates is 19.0 millimeters, the corrected value is 19.2 millimeters. Plot these corrected values, including the average of the 0.5 microgram per milliliter concentration, on two-cycle semilog paper, placing the concentration in micrograms per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the standard curve through these points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 0.5 microgram per milliliter standard;

a, b, d, e = Corrected average values for the 0.32, 0.40, 0.625, and 0.78 microgram per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 0.5 microgram per milliliter solution and three cylinders with the 0.5 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 0.5 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 0.5 microgram per milliliter value on the standard curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Sterility.* Use 500 milligrams, or the entire contents if less than 500 milligrams, from each immediate container tested, and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria, and use 50 milligrams in lieu of 500 milligrams in the test for yeasts and molds. Adjust the pH of the Sabouraud medium to 7.4 with NaOH.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution prepared by diluting the sample to 10 milligrams of novobiocin per milliliter with sterile, pyrogen-free U.S.P. saline T.S.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except use sterile U.S.P. saline T.S. as a diluent, and administer a test dose of 0.5 milliliter of a solution containing 4 milligrams of novobiocin per milliliter.

(5) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 141e.401(g) of this chapter.

(8) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Transfer the solution to a 200-millimeter tube, and determine the angular rotation in a suitable polarimeter, using sodium light or a 5893 Angstrom filter. Calculate the specific rotation on the anhydrous basis.

(9) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 1.0 milliliter of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should be developed in the benzene layer.

(10) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148j.2 Calcium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin is the crystalline calcium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 800 micrograms per milligram, expressed in terms of novobiocin on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 10 percent.

(iv) Its pH in a saturated aqueous suspension containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its specific rotation in an acid-methyl alcohol solution at 25° C. is not less than –50° and not more than –58°.

(vi) It demonstrates a positive color identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, specific rotation, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148j.1(b)(1), except prepare the stock solution of the sample by dissolving in 5 milliliters of absolute ethyl alcohol and then diluting with sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 1,000 micrograms (estimated) per milliliter. Dilute an aliquot of the stock solution with 1 percent potassium phosphate buffer, pH 6.0, to an estimated concentration of 0.5 microgram per milliliter.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except use sterilized U.S.P. saline T.S. as the diluent, and inject 0.5 milliliter of a solution containing the equivalent of 2 milligrams of novobiocin per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a saturated aqueous solution prepared by suspending 25 milligrams of calcium novobiocin per milliliter.

(5) *Specific rotation.* Proceed as directed in § 148j.1(b)(8).

(6) *Identity.* Proceed as directed in § 148j.1(b)(9).

(7) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148j.3 Sodium novobiocin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin tablets are tablets that contain sodium novobiocin, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each tablet contains 125 milligrams or 250 milligrams of novobiocin. The 125-milligram tablet contains 375 milligrams of sulfamethizole. Its moisture content is not more than 3 percent. The tablets

distintegrate within 1 hour. The sodium novobiocin used conforms to the standards prescribed by § 148j.1(a)(1)(i), (iv), (v), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., shall conform to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Sodium novobiocin used in making the batch for potency, toxicity, moisture, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency, moisture, disintegration time.

(ii) Samples required:

(*a*) Sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum of 30 tablets.

(*2*) For disintegration-time: Six tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(4) *Fees.* $0.75 for each tablet in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148j.1(b)(1), except prepare the sample by placing a representative number of tablets in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, to give a stock solution of convenient concentration. The content of novobiocin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148j.4 Calcium novobiocin-sulfamethizole tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin-sulfamethizole tablets are tablets composed of calcium novobiocin and sulfamethizole, with one or more suitable and harmless colorings, binders, diluents, and lubricants. Each tablet contains 125 milligrams of novobiocin and 250 milligrams of sulfamethizole. Its moisture content is not more than 10.0 percent. The tablets disintegrate within 1 hour. The calcium novobiocin used meets the standards prescribed by § 148j.2(a)(1) of this chapter. Each other substance used, if its name appears in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium novobiocin used in making the batch for potency, toxicity, moisture, pH, specific rotation, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The calcium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum of 30 tablets.

(*2*) For disintegration time: Six tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing not less than approximately 5 grams.

(4) *Fees.* $4.00 for each container in the samples submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph; $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Blend a representative number of tablets in a high-speed glass blender for 3 to 5 minutes with sufficient absolute ethyl alcohol to give a stock solution of convenient concentration. Proceed further as directed in § 148j.2(b)(1). The content of novobiocin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148j.5 Calcium novobiocin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin oral suspension is a suspension containing calcium novobiocin and one or more suitable and harmless diluents, preservatives, suspending agents, surfactants, flavorings, and colorings in purified water. Each milliliter contains 25 milligrams of novobiocin. The pH is not less than 6.0 and not more than 7.5. The calcium novobiocin used conforms to the standards prescribed by § 148j.2(a)(1)(i), (ii), (iv), and (vi). If sodium novobiocin is reacted with a suitable calcium salt to form calcium novobiocin, the sodium novobiocin used conforms to the standards prescribed by § 148j.1(a)(1)(i), (iv), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium novobiocin used in making the batch for potency, toxicity, pH, crystallinity, identity, and specific rotation. If sodium novobiocin is used in making the batch: Potency, toxicity, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The calcium novobiocin or the sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing not less than 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Remove a representative sample of the syrup with a suitable syringe, place into a high-speed glass blender with sufficient absolute ethyl alcohol to give a concentration (estimated) of 1,000 micrograms per milliliter. Blend for 3 to 5 minutes. Proceed further as directed in § 148j.2(b)(1). Its novobiocin content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted syrup.

PART 148o—PAROMOMYCIN

Sec.

148o.1 Paromomycin sulfate.
148o.2 Paromomycin sulfate capsules.
148o.3 Paromomycin sulfate syrup.

AUTHORITY: The provisions of this part 148o.3 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148o.1 Paromomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate is the sulfate salt of a kind of paromomycin or a mixture of two or more such salts. It is a creamy-white to light-yellow powder. It is so purified and dried that:

(i) Its potency is not less than 675 micrograms per milligram on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5.0 percent.

(iv) The pH of a 3.0 percent acqueous solution is not less than 5.0 and not more than 7.5.

(v) Its specific rotation at 25° C. in water is not less than +50° and not more than +55° on an anhydrous basis.

(vi) Its residue on ignition is not more than 2.0 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, specific rotation, and residue on ignition.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay using Staphylococcus epidermidis*—(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*1*) Make nutrient agar for maintaining the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

In lieu of preparing the medium from the individual ingredients specified in (*b*) (*1*) of this subdivision, it may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition of such medium. Minor modifications of the individual ingredients specified in (*b*) (*1*) of this subdivision are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(2) Use the nutrient agar described in this subsection for the seed layer and base layer, except that its pH after sterilization is 7.8 to 8.0.

(*c*) Working standard. Dry approximately 30 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the weight of the dried standard and dissolve in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration of 1,000 micrograms of paromomycin per milliliter. This stock solution may be kept in the refrigerator for 3 weeks.

(*d*) *Preparation of sample.* Dissolve the sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram per milliliter (estimated).

(e) *Preparation of test organism.* The test organism is *Staphylococcus epidermidis* (ATCC 12228), which is maintained on slants or agar described in (*b*) (*1*) of this subdivision. Wash the organism from the agar slant with 3 milliliters of sterilized U.S.P. saline T.S. onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in (*b*) (*1*) of this subdivision. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C.–35° C., and then wash the resulting growth from the agar surface with 50 milliliters of sterilized U.S.P. saline T.S. Standardize the suspension, so that a 1:25 dilution will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run plates to determine the quantity of the adjusted bulk suspension (usually 0.16 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(*f*) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in (*b*) (2) of this subdivision to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use plates the same day they are prepared. Melt a sufficient amount of agar described in (*b*) (2) of this subdivision, cool to 48° C., add the proper amount of the test organism as described in (*e*) of this subdivision, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1*M* potassium phosphate buffer, pH 8.0, to obtain concentrations of 0.64, 0.80, 1.00, 1.25, and 1.56 micrograms per milliliter. The 1.0-microgram per milliliter solution is the reference concentration. Use three plates for the determination of each point on the curve, except the 1.0-microgram per milliliter concentration, a total of 12 plates. On each of three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1-microgram determinations and 9 determinations for each of the other points on the curve. After the plates have incubated overnight at 32° C.–35° C., read the diameters of the circles of inhibition in the plates. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the point tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point of the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.80-microgram concentration, the average of 36 readings of the 1.0-microgram concentration were 20.0 millimeters, and the average of the 1.0-microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 0.80-microgram concentration of the same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 1.0 microgram per milliliter standard;

a, b, d, e = Corrected average values for the 0.64, 0.80, 1.25, and 1.56 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 1.0 microgram per milliliter solution and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.—35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the standard curve, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Plate assay using Bacillus subtilis.* As an alternative method, proceed as directed in § 141b.101 (a) through (i) of this chapter, except that:

(*a*) In lieu of the directions in § 141b.101(b) of this chapter, prepare the nutrient agar for the base and seed layers as follows:

Beef extract	6.0 gm.
Peptone	10.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.8–8.0 after sterilization.

In lieu of preparing the medium from the individual ingredients specified in (*a*) of this subdivision, it may be made from a dehydrated mixture which, when recon-

stituted with distilled water, has the same composition of such medium. Minor modifications of the individual ingredients specified are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(b) Prepare the working standard stock solution as directed in subdivision (i) (c) of this subparagraph, and in lieu of the concentrations for the standard curve as prescribed in § 141b.101(d) of this chapter, prepare concentrations of 1.28, 1.60, 2.00, 2.50, and 3.12 micrograms per milliliter from the stock solution and use 2.00 micrograms per milliliter concentration as the reference point.

(c) In lieu of the directions in § 141b.101(e) of this chapter, dilute the sample to a final concentration of 2.0 micrograms (estimated) per milliliter.

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer 0.5 milliliter of a solution of the drug in sterilized distilled water containing 8.0 milligrams per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 3.0 percent aqueous solution.

(5) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter volumetric flask. Dissolve in a few milliliters of water, add water to volume, and mix. Transfer the solution to a 200-millimeter tube and determine the angular rotation in a suitable polarimeter, using a sodium light or a 5,893 Angstrom filter. Calculate the specific rotation on an anhydrous basis.

(6) *Residue on ignition.* Place approximately 1 gram of the sample, accurately weighed, in a tared, metal-free procelain crucible, and add 2 milliliters of concentrated H_2SO_4. Completely char on low flame, raising the temperature gradually until fumes of SO_3 disappear. Ignite at a dull-red heat or in a muffle furnace at 1,100° F., cool, and weigh.

§ 148o.2 Paromomycin sulfate capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate capsules is paromomycin sulfate enclosed in a suitable and harmless gelatin capsule. Each capsule contains 250 milligrams of paromomycin. The moisture content is not more than 7.0 percent. The paromomycin sulfate used conforms to the standards prescribed therefor by § 148o.1(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The paromomycin sulfate used in making the batch for potency, toxicity, moisture, pH, specific rotation, and residue on ignition.

(b) The batch for potency and moisture.

(ii) Samples required:

(*a*) The paromomycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used: One package of each, containing not less than 5 grams.

(4) *Fees.* $0.75 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148o.1(b)(1), except prepare a stock solution of convenient concentration by pooling the entire contents of a representative number of capsules in an appropriate-sized volumetric flask and dissolve with 0.1*M* potassium phosphate buffer, pH 8.0. The paromomycin content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams that each capsule is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148o.3 Paromomycin sulfate syrup.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate syrup is a syrup that contains paromomycin sulfate and one or more suitable and harmless solvents, flavorings, colorings, preservatives, and buffers in water. It contains a quantity of sucrose sufficient to make a syrup. Each milliliter contains 25 milligrams of paromomycin per milliliter. Its pH is not less than 7.5 and not more than 8.5. The paromomycin sulfate used conforms to the requirements of § 148o.1(a)(1)(i), (ii), (iv), (v), and (vi). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays for:

(*a*) The paromomycin sulfate used in making the batch for potency, toxicity, pH, specific rotation, and residue on ignition.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The paromomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing not less than 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(iii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148o.1(b)(1), except prepare the sample for assay by removing an appropriate aliquot of the syrup with a suitable syringe and transfer to an appropriate volumetric flask. Dilute to volume with distilled water and mix well. Make proper estimated dilutions of an aliquot to the prescribed reference point with 0.1*M* potassium phosphate buffer, pH 8.0. The potency is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of paromomycin that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

It is not deemed necessary to provide notice of proposed rulemaking in the case of the two new antibiotic drugs not included in the notice published in the FEDERAL REGISTER May 2, 1963, since these are formulations of the basic antibiotic drug included in the proposal.

Effective date. This order shall become effective 60 days from the date of its publication in the FEDERAL REGISTER.

(Sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357)

Dated: June 4, 1964.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 64-5746; Filed, June 12, 1964; 8:49 a.m.]